AN ABSTRACT OF THE THESIS OF

John Hayden for the degree of Master of Science in Soil Science presented on November 12, 1999. Title: Low Molecular Weight Organic Acids in Forest Soils.

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John E. Baham

Two studies concerning low molecular weight organic acids in soils were conducted. In the first study, anion exclusion chromatography was used to separate and identify 12 common organic acids, and the accuracy, precision and detection limits of the method were determined. The method was found to be sensitive and accurate to between 1.5 and 9 μM, depending on the acid in question. The stability of the selected acids was examined, and the large concentration changes observed underscore the importance of timely analysis of soil solutions. No significant interferences were encountered in the analysis of two soil solutions. In the second study, the reaction of a forest soil with oxalic acid at four concentrations was monitored for a period of 96 hours. A large release of aluminum, sulfate, and phosphate was observed, with the greatest release occurring with the highest concentration of added oxalate. Solution aluminum increased by up to a factor of twenty, and though $[Al^{3+}]$ values were consistent with control by thermodynamic equilibrium with an amorphous aluminum oxide phase, exchangeable Al appeared to be the source of the

increase. Sulfate increased abruptly at the start of the reaction and continued to rise, though more slowly, throughout the study. Solution phosphate was increased by up to four times and was maintained at the elevated level throughout the study. Changes in both sulfate and phosphate concentrations were attributed directly to exchange with oxalate. The persistence of elevated phosphate concentrations after 96 hours indicates that the effects of oxalate production by mycorrhizae could have lasting effects on the nutrient status of a soil.

Low Molecular Weight Organic Acids in Forest Soils

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LOW MOLECULAR WEIGHT ORGANIC ACIDS IN FOREST SOILS

CHAPTER 1

INTRODUCTION

Low molecular weight organic acids are important in the chemistry of soils, and are especially abundant in forest soils. The ability of low molecular weight acids to form complexes with metals affects the solubility of soil solids, and the solution activity and bioavailability of elements such as Fe, Al, P, Cu, and Ca. Organic acids are also important in the chemical mass balance of soils, sometimes increasing and sometimes retarding the flux of nutrients from the soil.

Organic acids are derived from the decomposition of plants, animals, and microorganisms, as well as from the wastes and exudates produced by living organisms (Bar-Yosef, 1992). Organisms are involved in evolutionary adaptation with their environments, and the effects of these acids on the soil have immediate consequences for soil organisms. A first requirement for understanding these effects are reliable analytical methods for the identification and quantification of the chemical components of the soil solution. The second chapter in this thesis is an evaluation of a chromatographic method for the determination of low molecular weight organic acids. In addition, some attention is given to the determination of aluminum in soil solutions, in appendix A.

The third chapter is a laboratory investigation of the effects of a single organic acid, the oxalate anion, on a forest soil sample, with specific attention to the dynamics of aluminum and phosphorous. Oxalate was

chosen because it is produced in large quantities by mycorrhizal fungi associated with coniferous forest trees, and it has been proposed that this modifies the soil environment to the benefit of both mycorrhizal partners. An attempt is made to discover the mechanisms of the observed changes in the soil solution.

CHAPTER 2

ANALYTICAL DETERMINATION OF LOW MOLECULAR WEIGHT ORGANIC ACIDS IN SOIL SOLUTIONS BY ANION EXCLUSION CHROMATOGRAPHY

ABSTRACT

Low molecular weight organic acids are important components of soil solutions, and before quantitative studies of their role in soil processes can be made, accurate and reliable methods for organic acid analysis are required. Organic acids are extremely susceptible to photo-oxidation and other degradative processes, and therefore knowledge of their stability during storage is imperative. In this study, anion exclusion chromatography was used to separate and identify eight common organic acids which occur in soil solutions of forested and agricultural soils. Values for precision, accuracy and detection limits were determined for a series of mixed acid standards. Both the precision and the accuracy of the analysis increased with organic acid concentration. At 250 μM in the mixed acid standard, relative precision was better than 4% for all acids, except for oxalic. Relative precision values exceeded 100% in some cases for analysis at concentrations below 2.5 μM. The decrease in precision at the lower concentration was the basis for the determination of detection limits. Analysis of three soil solutions by comparison of the peak areas to linear calibrations, and by the method of standard additions, are in reasonable agreement for the acids which could be identified. The low molecular weight organic acids comprise from 5-15% of the dissolved organic carbon

in the soils analyzed. No storage conditions were found to prevent changes in concentration of organic acid standards. As much as 50% degradation was found to occur within 8 days. The least degradation occurred in those standards stored in the dark under refrigeration. The low concentration of organic acids in soil solutions and their short lifetime places a premium on timely analysis.

INTRODUCTION

Low molecular weight aliphatic acids are involved in many biogeochemical and weathering processes in soils (Stevenson, 1974). A number of these acids occurring in the soil solution (e.g. citric and oxalic) form soluble complexes with many metals, including iron and aluminum. This can ameliorate Al toxicity to plants, induce solid phase dissolution through the depression of solution ion activities, and facilitate the movement and leaching of metals. The ability of these organic acids to form surface complexes with oxides, primary minerals, and secondary phosphorous containing solids (Stumm, 1986; Ochs, et al., 1993) destabilizes these solids, enhancing mineral weathering and nutrient release to solution (Bar-Yosef, 1992, Hue et al., 1986). Despite the fact that organic acids are present at relatively low concentrations, they are a dominant force in the weathering of soils and appear to be an effective plant strategy for obtaining P (Ochs, 1993).

The reaction of the organic acids with the mineral phase of soils and the ensuing pedochemical weathering clearly illustrates a link to the transfer of energy between the biotic and geochemical compartments of

terrestrial ecosystems. The production of organic acids in soils is ultimately the result of photosynthesis at higher trophic levels; however, these acids can be produced by bacteria and, in particular, by fungi and mycorrhizae associated with higher plants. Below-ground processing of carbon and the formation of organic acids is thought to represent a significant fraction of the net primary productivity of forest ecosystems, but only limited information is available regarding the temporal fluxes of organic acids in soil ecosystems.

The complexity of these fluxes in soils is illustrated in a study of the Spodosol soil solutions during a growing season (Krzyszowska et al. 1996). Dissolved organic carbon concentration increased slowly in the first part of the season followed by a decline to the original values. However, individual organic acids did not folow this pattern. Oxalic acid concentration was elevated until midsummer, when it abruptly declined; formic acid showed a brief increase in concentration in late summer; while acetic acid concentration fluctuated throughout the season. Acetic acid also showed great spatial variablity, which was attributed to soil moisture content, microbial activity, and microclimate.

Recent interest in Al toxicity, acidic deposition, and pedogenesis has fostered renewed interest in the quantification of organic acids in soil environments (Fox and Comerford, 1990; Hue et al., 1986; Pohlman and McColl, 1988). The dominant acids which have been identified include: oxalic, citric, formic, acetic, malic, lactic, maleic, malonic, fumaric, aconitic and succinic acid (Fox and Comerford, 1990; Hue et al., 1986; Smith 1967; Pohlman and McColl, 1988, McColl and Pohlman, 1986; Bruckert, 1970).

Typical concentrations of organic acids in mineral soils and leaf litter extracts are on the order of 1-1,000 μM on a soil solution basis. The total concentration of organic acid represents from 2 to 10 percent of the DOC (Fox and Comerford, 1990). Oxalic acid is often the dominant acid detected in soils. Soil solutions extracted from soils colonized by fungal mats may in some cases have oxalate concentrations (6 mM) which exceed the solubility of calcium oxalate (Griffiths et al., 1994).

The above discussion points to a critical need for study of the fluxes and reactions of organic acids in soil and terrestrial ecosystems, and this will require reliable measurements of the concentrations of individual acids. Because most of these acids occur at concentrations approaching the limit of detectability of present analytical methods, it is important to determine the precision, accuracy, and detection limits of any method employed. Another important factor in organic acid analysis is analyte stability during sample handling and storage. The goal of this work is to evaluate the precision, accuracy and detection limits of a chromatographic method for a suite of commonly occurring organic acids found in soils, and to explore the effect of storage time and conditions on the stability of low concentration standards.

MATERIALS and METHODS

Organic Acids

A suite of organic acids were selected for analysis taht are common in soil solutions or leaf litter extracts (Hue et al., 1986; Fox and Comerford, 1990; Pohlman and McColl 1980; Smith 1969; and Bruckert, 1970).

Aqueous stock solutions of the individual acids (1 *mM*) were prepared from pure acid salts or liquids. Individual standards (10, 100, and 500 *μM*) and mixed standards (500, 250, 100, 75, 50, 25, 1, 5, 2.5, and 0.0 *μM*) containing the eight representative acids which could be resolved by anion exclusion chromatography were prepared for analysis.

Chromatography

Separation and quantification of the soluble organic acids was made on a Dionex 2000i Ion Chromatography System (Dionex, Corp., Sunnyvale, CA) by anion exclusion employing a conductivity detector (Dionex Corp., Sunnyvale, CA). Separation of the organic acids was made isocratically with a ion exclusion column (Alltech-Wescan Anion Exclusion Column 300 x mm and Guard Column; Alltech Corp., Chicago, IL) using 1.8 *mM* H_2SO_4 as the eluant at 0.6 mL m^{-1}. Samples were loaded from a 20 μL sample loop. Post separation ion suppression was made with a micromembrane ion suppressor (Dionex, Corp., Sunnyvale, CA) using 2.5 *mM* tetrabutylammonium hydroxide at 2 mL m^{-1} as the regenerant. The chromatograms were stored on floppy disk and analyzed with Dionex AI-400 Ion Chromatography software (revision 07; Dionex Corp., Sunnyvale, CA).

Retention Times

Determination of the chromatographic retention time for a single organic acid was made by running duplicate chromatograms at 10, 100,

and 500 *μM*. The retention times were used as a criteria in the selection the of the acids for the mixed standard.

Detection Limits, Precision and Accuracy

Linear calibration plots were generated by the analysis of the mixed organic acid standards. Mixed acid standards containing 0.0, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, 100, and 500 *μM* of each of eight acids were analyzed five times. A conservative measure of the detection limit for an individual acid was estimated by extrapolation of the standard linear calibration to zero concentration. To assess precision, calibration standards of known concentration ranging from 2 to 250 *μM* were analyzed in quadruplicate and the values obtained from the standard ion chromatogrphy (IC) method were compared with the known concentrations.

To assess the accuracy of the method, soil solution extracts were analyzed by comparison with external standards and by the method of standard additions (MSA). Soil samples were collected from a Pacific Northwest coniferous forest site in Oregon, some from soils colonized by the ectomycorrhizal fungi *Gautieria monticola* and *Hysterangium setchellii*, and some from nearby uncolonized soils. The field moist samples were passed through a 2 mm sieve and the soil solutions (1:1 water-soil) were obtained by centrifugation. For MSA analysis, the extracts were "spiked" with 5, 12.5, and 25 *μM* mixed organic acids. The response of the IC method to the known additions of each acid was used to calculate the concentration of the acid in the unspiked extract.

Stability of the Organic Acids in Mixed Standards

The stability of mixed organic acid standards under different storage conditions was investigated by preparing a mixed solution of eight acids. An aliquot of the mixed acid standard (25 *μM*) was dispensed into nine plastic bottles. Three bottles were placed in each of three storage conditions: i) dark under refrigeration; ii) dark with no refrigeration; and iii) laboratory bench under ambient conditions of light and temperature. Concentrations of the organic acids were determined by IC after 1, 3, 6, and 8 days.

RESULTS and DISCUSSION

Retention Times

Acid concentration had little effect on chromatographic retention time or peak width (Fig. 2.1). Typical peak widths are approximately 25 seconds, limiting the resolution to acids which have retention times which differ by greater than 35 seconds. A downward shift in the retention time of an individual acid appeared to take place when actual soil solutions were analyzed. Evidently, this was the result of a reversible fouling of the column which could be remedied by routine cleaning of the column. Adjustment of the eluant concentration from 3.0 *mM* to 6.0 *mM* H_2SO_4 had little effect on the retention times. However, eluant concentration, flow rate and column temperature might be successfully controlled to enhance separation and run times. Gradient elution with acetonitrile may also be

employed to enhance separation and reduce analysis time (Lee and Lord, 1986).

Oxalic acid eluted near the solvent front of the exclusion column. Resolution of oxalic acid in solutions containing significant amounts of sulfate and chloride may be difficult since these components may co-elute

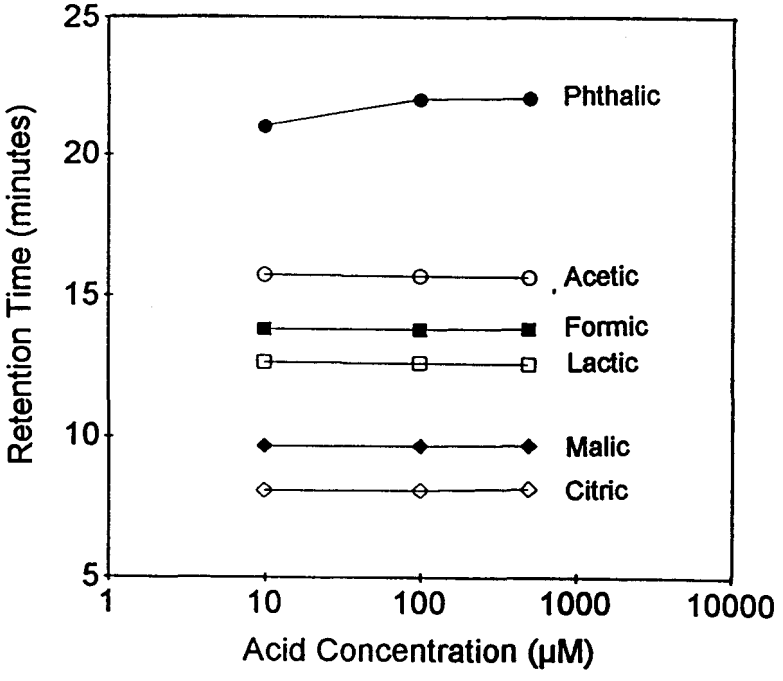

Fig. 2.1. Retention times of individual acids.

with oxalate. This is one possible disadvantage of conductivity detection relative to UV detection, which is more selective for organic chromophores.

Precision

The precision of analysis for an individual component of an organic acid mixture is a function of the concentration (Fig. 2.2). Precision improved for all of the acids at higher concentrations. The coefficient of variation of the five repetitions ranged from 85% for oxalic acid at

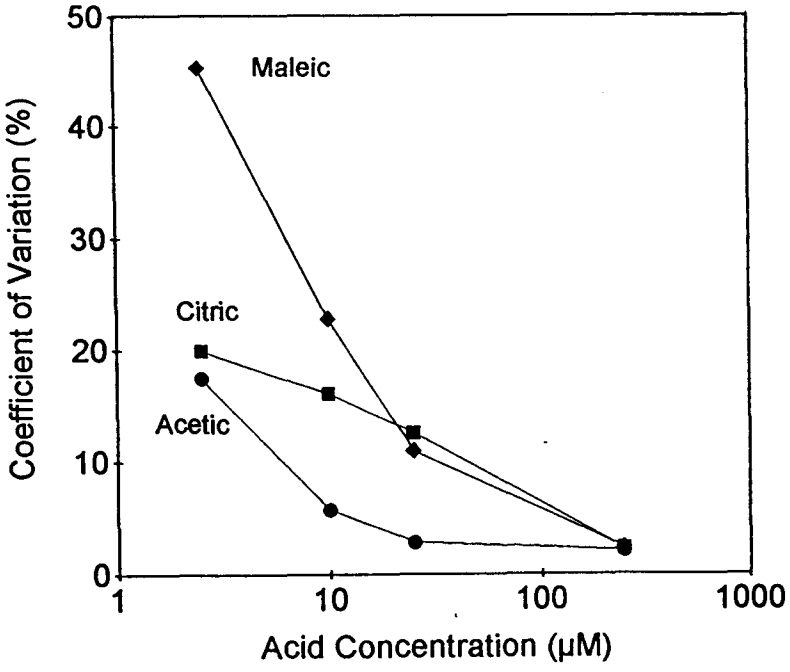

Fig. 2.2. Precision of the analysis at various concentrations.

concentrations near the detection limit, to less than 1% for the analysis of check samples of formic and lactic acids at concentrations greater than 250 μM. These values for precision are comparable to estimates obtained for oxalic, formic and acetic acids extracted from soils by Fox and Comerford (1990) and Krzyszowska et al. (1996). The precision is generally better than 15% at concentrations at or above 10 μM.

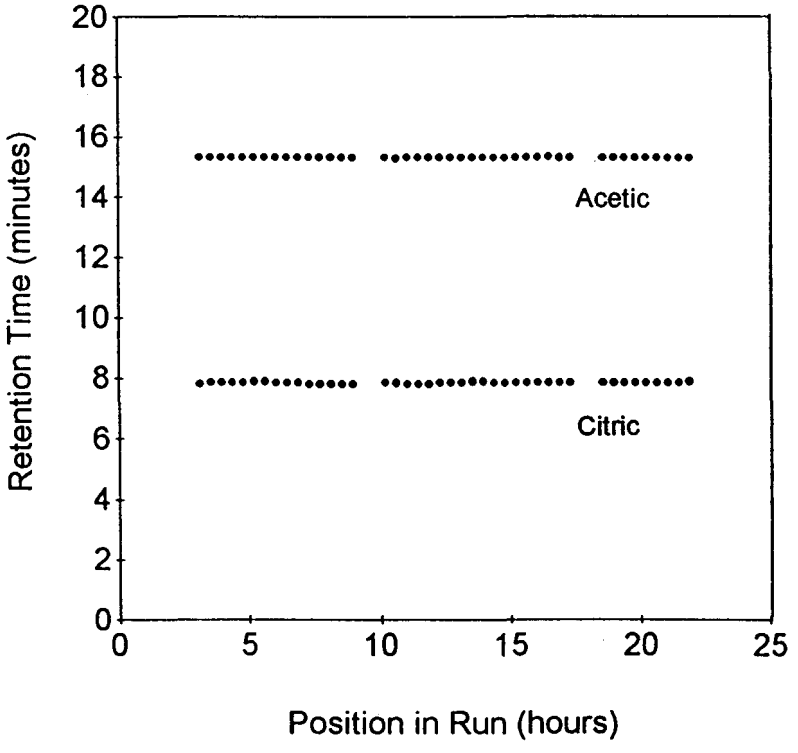

Fig. 2.3. Retention time stability for selected acids during an analytical run.

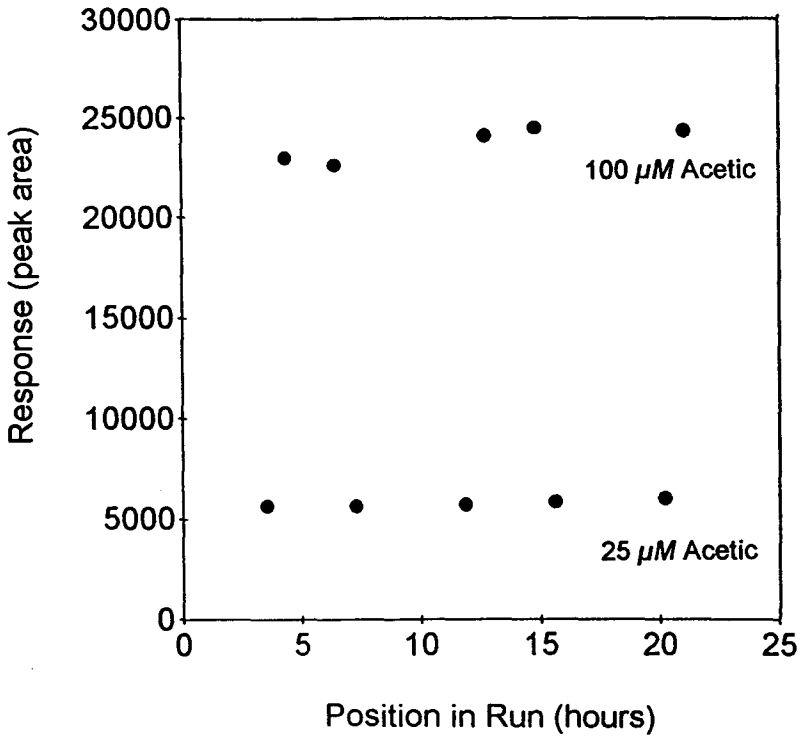

Fig. 2.4. Stability of instrument response to two acetic acid standards during an analytical run.

Analytical Performance

No change in retention time for individual acids was found during the course of a 24 hour analytical run composed of nine different standards (Fig. 2.3). During the course of a given analytical run,response to all acids changed less than 8% (Fig. 2.4). This data indicates that the analytical system is stable over time. Figure 2.5 presents the chromatogram of a solution extract spiked with 25 *μM* of each analyte.

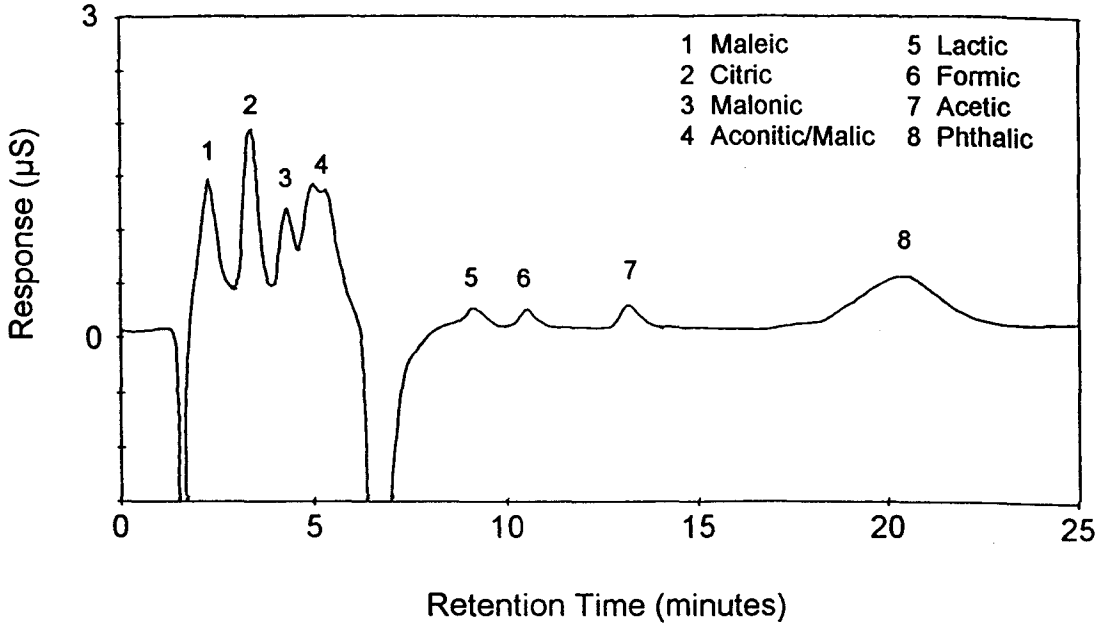

Fig. 2.5. Chromatogram of a soil solution spiked with eight organic acids at 25 *µM*.

Detection Limits

Conservative estimates for the detection limits were obtained by extrapolation of the linear calibration to zero concentration. The range of the calibration line included 24 standards (0, 1, 2.5, 5, 10, 25). Detection limits for each acid were determined by plotting 95% confidence limits for the prediction of concentration from the instrument response (Fig. 2.6). Point M represents the lowest instrument response from which a non-zero acid concentration can be predicted with 95% confidence. The concentration at point C is thus the reported detection limit. Detection limits for selected organic acids are presented in Table 2.1.

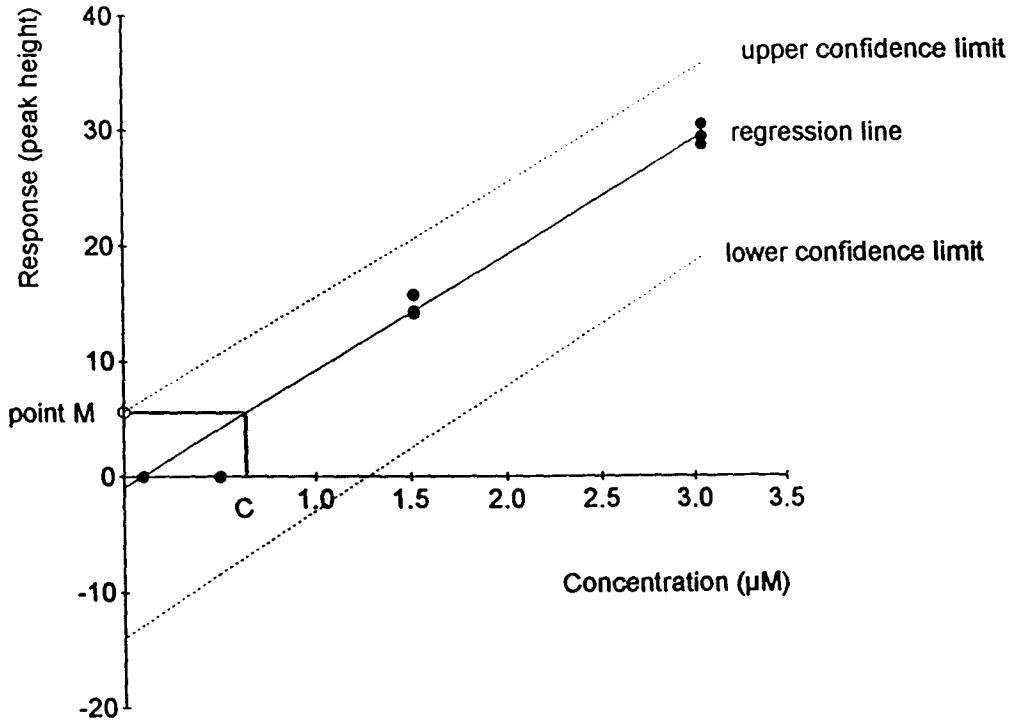

Fig. 2.6. Determination of a detection limit from the lower end of a standard calibration curve (where point C is the detection limit).

Table 2.1. Detection limits for selected organic acids.

Acid	Detection Limit (μM)
Citric	2.5
Maleic	3.4
Lactic	1.5
Malonic	1.4
Formic	1.3
Acetic	2.0
Phthalic	1.8

Standard Stability

Storage conditions and time had a marked effect on the concentrations of many of the acids in the 25.0 *μM* mixed standard. The concentrations of oxalic, citric, malonic and malic acids declined as much as 50% with time regardless of the storage conditions. Surprisingly, maleic acid concentrations actually appeared to increase over 8 days, while the concentration of oxalic acid first increased 30% over three days and then declined to approximately 50% of its original value after 8 days. The concentration of a 25 *μM* formic and acetic acids in a mixed acid standard remained constant when stored in the dark, but decreased in ambient lighted conditions in the laboratory (Fig. 2.7). No obvious biological activity or turbidity was observed in any of the standards over the 8 day stability experiment. Oxidative and photochemical reactions were undoubtedly responsible for the apparent degradative loss of analyte signal from standards containing low concentrations of organic acids. It is recommended that standards be prepared daily from concentration stock solutions and that all samples and standards be stored under dark refrigeration prior to analysis.

Accuracy

Results obtained from the method of standard addition for three soil solutions are compared to concentration values estimated from comparison to linear calibration techniques of mixed standards (Table 2.2). Background effects and other interferences presumably are overcome with

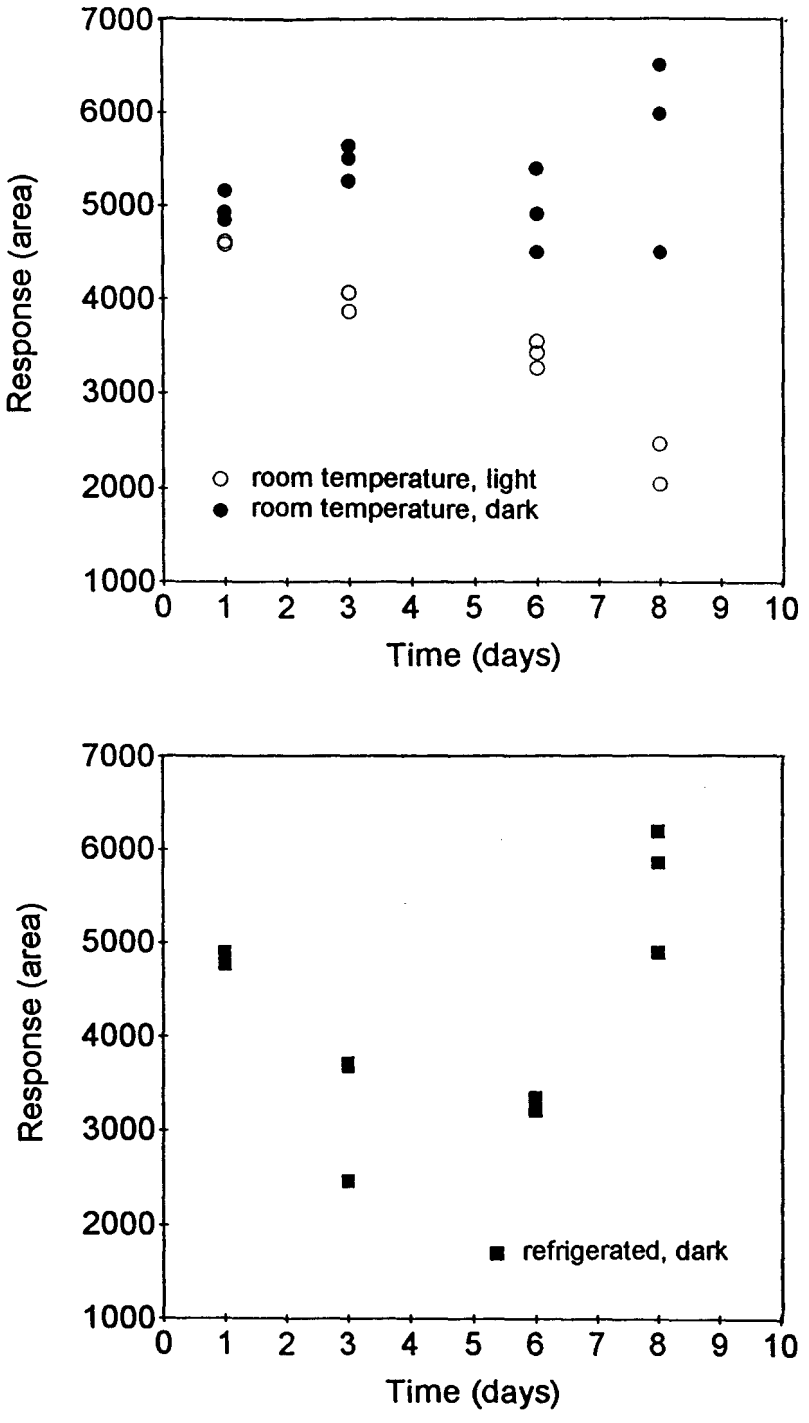

Fig. 2.7. Stability of 25 μM acetic acid standard under three storage conditions.

Table 2.2. Accuracy of soil solution analysis obtained by two methods: comparison to external standards and the method of standard additions (in μM).

	Calculated by Standard Curve			Calculated by Standard Additions
	replicates		mean	
Uncolonized Soil				
Maleic	24.8	22.5	23.6	16.7
Citric	2.5	2.5	2.5	2.1
Gautieria Mat Soil				
Maleic	7.0	6.2	6.6	5.7
Citric	5.8	5.9	5.8	5.4
Phthalic	68.7	65.9	67.3	10.3
Aconitic/Malic	9.7	9.4	9.7	8.6

the method of standard additions. Although the concentrations obtained by the two methods are not in perfect agreement, it is reasonable to conclude that the chromatographic response to acid concentrations in a mixed standard roughly approximates the behavior of acids in a more complex mixture, i.e. a soil solution.

CONCLUSIONS

Anion exclusion chromatography is a precise, accurate, and sensitive method for the determination of low molecular weight aliphatic acids in soil solutions. A number of organic acid are present in soil solutions in detectable concentrations. Caution should be exercised in the preparation and storage of soil solutions and mixed acid standards. At low concentrations (25 μM) aliphatic acids apparently undergo a set of rather rapid light and/or O_2 induced chemical reactions, resulting in measurable changes in analytical concentrations of the acids. Because organic acid concentrations will begin to change as soon as a soil is sampled due to altered production and consumption rates, attention to timely analysis is of great importance to the accurate reporting of their concentrations in soils.

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CHAPTER 3

OXALATE-INDUCED PHOSPHORUS SOLUBILITY IN A FOREST SOIL

ABSTRACT

Oxalate is produced by many groups of mycorrhizal fungi in association with forest trees. This study was designed to establish the effects of oxalate on the soil solution chemistry of aluminum and phosphorus. Soil samples from the A horizon of a slightly acid forest soil were reacted with 100, 500, and 1000 *μM* oxalate at a 1:10 soil:water ratio for 96 hours in a laboratory study. Reaction kinetics were observed by periodic analysis of the aqueous solution for aluminum, phosphate, pH, oxalate, sulfate, and nitrate. Solubilization of aluminum was closely correlated with oxalate concentration during the reaction period. Monomeric aluminum concentration was increased from less than 2 *μM* to as much as 190 *μM*. Phosphate concentration was increased by the addition of oxalate from less than 1 *μM* to as much as 4 *μM*, and remained elevated for the duration of the study. Results indicated that the solution phosphate was increased through ligand exchange with oxalate. Ion activity products (IAP) were consistent with solution-aluminum control by solid phase $Al(OH)_3$, but the primary source of solubilized aluminum was considered to be exchangeable aluminum. Propylene oxide vapor was used as a biostat and was apparently effective in reducing oxalate metabolism during the reaction. This treatment increased phosphate concentration from 1 *μM* to 3 *μM*, and reduced soil pH from 5.8 to 5.4.

INTRODUCTION

Mycorrhizae

In the mid-elevation mountain forests of Oregon, a major limitation to the growth of trees is the low concentration of phosphorus in the soil solution, due to an abundance of volcanic ash-derived aluminum and iron (Entry et al.,1991). These metals form solid hydroxide phases with great P-sorbing ability, and can also precipitate with phosphate as more or less crystalline aluminum- or iron-phosphates. Another concern in many of these soils is high concentrations of aluminum in the soil solution that can be toxic to many organisms.

In these forests, the predominant tree species, douglas fir (*Pseudostuga menziesii*), is often associated with one of a number of mycorrhizal fungi, including *Hysterangium crassum*, *H. setchellii,* and *Gautieria monticola*. Similar mycorrhizal associations are found in California, New Mexico, and Connecticut (Graustein et al., 1977), and in North Carolina. Coniferous mycorrhizal associations with other fungal species are found world-wide and are an essential part of most temperate forest ecosystems (Kropp and Langlois, 1990). Research has established the benefit to forest trees from these associations, and the intentional inoculation of tree seedlings with mycorrhizal fungi purposes has become widespread (Stenstrom and Ek, 1990).

Much has been learned about the use of mycorrhizae in the production of tree seedlings for reforestation purposes. It has been found that high-phosphate growing conditions are unfavorable for the establishment of the mycorrhizae, and in these situations the fungal partner

may be a drain and a hinderance to the tree (Browning and Whitney, 1993). Also the correct matching of the partners is important (Kropp and Langlois, 1990). Mycorrhizae are known to increase plant uptake of P from the soil, and their fungal hyphae are able to transport P by an active process (Skinner and Bowman, 1974). Very little is known, however, of the mechanism of these effects. This knowledge could be very useful in light of the possibility that mychorrhizae could be genetically improved, just as trees are bred today.

The benefit to trees of mycorrhizae has been thought to be mostly physical, including improved water relations and protection from pathogenic organisms. Some attention has been given to chemical effects, most of which involve the production of compounds that interact, directly or indirectly, with nutrients in the soil. One striking characteristic of the mycorrhizal mats in Oregon suggests a possible chemical mechanism. Large accumulations of calcium oxalate, often present as macroscopic crystals, are found in these mats (Cromack et al., 1979; Entry et al., 1991), and the fungi are presumed to be the source of the oxalate. The strong affinity of the oxalate anion to form complexes with aluminum suggests that it will favorably affect the solubility of phosphorous (Lan et al., 1998; Bhatti et al., 1998b). If oxalate can complex aluminum to the extent that aluminum is unable to react with phosphate, then solution orthophosphate may be increased (Iyamuremye and Dick, 1996; Bhatti et al., 1998a).

The purpose of the present study is to investigate the effect of this compound, oxalic acid, on the orthophosphate concentration in a soil solution. This is an *in vitro* recreation of a mycorrhizal system without the

effects of nutrient uptake, root exudates, root-induced pH changes, and other biochemical factors.

The major questions in the present study are: i) how does oxalate added to a soil affect solution phosphate?; and ii) what is the mechanism of this effect?. This study will focus on the dynamics of aluminum, leaving the effects of soluble iron to future work. Because the amount of aluminum in these soils greatly exceeds the amount of phosphorous, the behavior of aluminum is essentially independent of the soil P dynamics. Therefore, the first consideration will be aluminum.

Aluminum

The solution activity of Al^{3+}, the hexaquoaluminum ion, reflects the quantity of dissolved aluminum, and can be related by thermodynamic and kinetic theories to both solid and adsorbed forms of aluminum.

The simplest aluminum solid that can be considered to control (Al^{3+}) is gibbsite, $Al(OH)_3$, or amorphous aluminum hydroxide, $Al(OH)_{3(amorph)}$. Lindsay (1979) describes a method that gives (Al^{3+}) as a function of pH, using thermodynamic data for the dissolution of gibbsite (or amorphous $Al(OH)_3$). The free energy of the dissolved components of the solid will be determined, at equilibrium, by the structure and composition of the solid. Crystalline gibbsite has a lower molar free energy than does its amorphous analogs and therefore supports a lower aggregate component activity in solution. This component activity is quantified as the ion activity product (Sposito, 1989).

When silicon and other elements are introduced, more solids are possible, each of which will control (Al^{3+}). The Guy-Lussac-Ostwald step

rule states that (Al^{3+}) is determined by that solid that sets (Al^{3+}) at the highest activity. Lindsay (1979) considered $Al(OH)_3$, montmorillonite, and kaolinite as possible Al-controlling solids. Hydroxy-aluminum clay interlayers have also been considered as a possibility (Turner and Brydon, 1965; Bloom et al., 1977), as have allophane and imogolite (Manley et al.,1987).

Organic matter has appreciable effects on solid-solution relations. Aluminum binds strongly to many organic molecules, including low molecular weight acids, fulvic, and humic acids (Hargrove, 1986) In the precipitation of aluminum containing solids, this leads to the incorporation of organic molecules and the consequent malformation of the crystal structure (Ng Kee Kwong and Huang, 1975, 1977; Goh and Huang, 1984). This slows the precipitation rate and increases the P-sorbing capacity of the solid (Ng Kee Kwong and Huang, 1978). The ability of organic acids to increase the rate of the dissolution of many minerals is well known (Mast and Drever, 1986; Chou and Wollast, 1984). This may be due to the depression of (Al^{3+}) by complexation, or, more significantly, to the formation of activated Al complexes on the surface (Furrer and Stumm, 1986; Ochs, 1993). This complexation phenomenon is closely related to the often noted ability of organic acids to increase the solubility and mobility of Fe and Al in soils (McColl and Pohlman, 1986).

Aluminum also exists as exchangeable species adsorbed to soil surfaces. Exchange reactions are much faster than mineral precipitation/dissolution (Walker et al., 1988), and thus the position of the exchange is set by the solid phase. A short term perturbation of (Al^{3+}) from

this equilibrium position by a change in the soil solution will have an immediate effect on the exchangeable pool, and then as equilibrium is restored by precipitation (or dissolution) of the controlling solid, the exchangeable fraction will return to its former value. During the perturbation, the Al^{3+} in the solution and in the exchangeable phase fit an adsorption isotherm kinetic model. This has been suggested as part of the cause of periodic declines in total soluble Al in streams during rainfall events (Hooper and Shoemaker, 1985). Normally in equilibrium with a solid phase, labile solution- and exchangeable-Al is removed from the soil too quickly for dissolution of the controlling solid phase to maintain the equilibrium.

In organic soil horizons with large amounts of organic matter, (Al^{3+}) may be controlled by exchange on organic compounds (Bloom, 1979). (Al^{3+}) can be much lower than would be expected only on the basis of solid/solution equilibrium because of the very high Al-adsorption capacity of the organic matter. The carbon content of the present soil is low enough that this mechanism will not be considered as a factor.

Phosphorus

Orthophosphate (ortho-P) activity in the soil solution may also, like aluminum, be controlled by solid/solution equilibria or by exchange equilibria. In moist temperate forest soils, the major exchange phase for ortho-P is aluminum hydroxide,with with it reacts by ligand exchange mechanism (Sposito, 1989). The less well structured the aluminum hydroxide, the greater is P sorption (Sims and Ellis, 1983). This may be due to a greater specific surface area or , more likely, to a greater number

of non-bridging oxygens in the structure (Ng Kee Kwong and Huang, 1978). It has already been mentioned that incorporation of organic anions into the aluminum hydroxide has this same destructuring effect.

Orthophosphate competes with other anions for adsorption on solid soil phases, and its great affinity for adsorption significantly reduces P-availability to plants. That the great bulk of P is not in solution is detrimental to plant nutrition, but it is beneficial in so far as phosphate is not lost to leaching. If a plant is able to temporarily and locally increase solution-P, then it will have both adequate P nutrition and a stable, relatively unleachable reservoir of P.

Organic anions produced during decomposition by biotic activity (Pohlman and McColl, 1988) and by exudation from plant roots and mycorrhizae (Fox and Comerford, 1990) compete with orthophosphate for adsorption sites. Though their adsorption affinity is less than that of ortho-P, the large amounts of these anions that can be produced can displace a significant amount of ortho-P (Lopez-Hernandez et al., 1986; Nagarajah et al., 1970). This effect varies with the type of organic anion; some have no competitive ability (Appelt et al., 1975). The relative competitive ability of ortho-P and organic anions is influenced by which of the species is initially adsorbed. P is more adsorbed when it is added before oxalate, and less adsorbed when oxalate is added first (Violante et al., 1991).

Organic anions can affect the adsorption of ortho-P by acting on the exchanger phase directly. Their role in accelerating the dissolution of Al-containing solids was mentioned earlier, and this accounts for the decrease in P-sorption observed in some systems (Earl et al., 1979).

Extensive studies have been done on the adsorption of P by soils, and have often been interpreted by adsorption isotherms. However, P-sorption is often not fully reversible, and a desorption isotherm is usually different from the initial adsorption isotherm. Ortho-P has been observed or postulated to have: diffused into the interior of the adsorbent phase (McLaughlin et al.,1977; Ryden et al.,1977); formed a separate phase (Kittrick and Jackson, 1954, 1955; Low and Black, 1950; Vieth and Sposito, 1977), or a separate Fe-P phase (Martin et al., 1988); and to have entered a clay interlayer Al-OH-P phase (Weber and Clark, 1969; Weber, 1978). These results suggest that short term changes in solution orthophosphate may be due in part to solid/solution equilibria.

The mechanism of adsorption, as described by Sposito (1989), is very similar to the process of crystallization. Ligand exchange is the replacement of covalently bonded OH^- with orthophosphate, the phosphate's oxygens reforming the covalent bond with aluminum. This results in much stronger sorption than is involved in the electrostatic adsorption of other anions. A significant feature of crystallization is the repeatability of the bond-creating step, which always culminates in the recreation of an earlier configuration, and thus in the possibility of continued addition of structural units. This is true of covalent crystals as well as of ionic crystals. When adsorption phenomena are examined, it is seen that the mere electrostatic adsorption of ions to a solid surface does not result in a basis for further adsorption; at most an electric double layer is formed, which is unable to extend itself far from the surface. However, when a phosphate anion is bound to an aluminum hydroxide surface in an

inner sphere complex (that is, by ligand exchange), there is a very real possibility of the addition of a new monomeric aluminum ion to the phosphate. This is then the initiation of a crystal growth process. The crystal structure of the new Al-phosphate will be different from that of the original Al-hydroxide, yet continuous covalent bonds will join the two domains.

Ligands that can coordinate with aluminum include H_2O, OH^-, $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}. Thus the range of possible aluminum-phosphates is quite large, and attempts to characterize soil forms have met with little success. Pure, well structured aluminum phosphate (variscite) has never been found in soils (Hsu, 1982), however laboratory studies have yielded amorphous Al-phosphates ($Al^{3+} + PO_4^{3-} = AlPO_4$) with pK_{sp}'s ($[Al^{3+}][PO_4^{3-}]$) between 19 and 21 (Cole and Jackson,1960; Traina et al.,1986). Al-phosphates can also form in interlayers rather than as discrete minerals (Weber, 1978).

Orthophosphate can also be a minor constituent in amorphous $Al(OH)_3$ materials. This case is especially likely in view of the relative concentrations of the two elements in soils. In order to determine a solubility product for this type of solid, the percentage of phosphate substitution must be specified, and will of course be highly variable in soils. Jameson and Salmon (1954) investigated the solid/solution relations of the system aluminum oxide-phosphoric acid-water, in order to determine the solubility and phase relationships of various Al-phosphates. Though their phase diagram is not useful for soil systems because of the large

concentrations of soluble phosphate used in their experiments, the principle is well illustrated.

The Present Study

The objective for the present experiment is to determine the effects of oxalic acid on soil solution chemistry and to elucidate the important reactions involved. This will be done by reacting a soil with various concentrations of oxalate and measuring solution parameters over a four day period.

MATERIALS AND METHODS

Collection/Site

Soil samples were obtained from the H.J. Andrews Experimental Forest near Blue River, OR. The site was on the shoulder of a low ridge, with 10% slope, and the vegetation was predominantly Douglas fir (*Pseudotsuga menziesii*) with younger western hemlock (*Tsuga heterophylla*) and western yew (*Taxus brevifolia*). The upper two horizons, to a depth of 32 cm, were used for the experiment, because this is where the mycorrhizae occur and where the major effect of oxalate on phosphorous availability would take place. The soil used was not colonized by mychorrhizae. Soil samples were passed through a 2-mm sieve, and kept refrigerated in plastic bags until the experiment.

Sterilization

In order to reduce microbial activity during the 96 hour reaction time, the soils were exposed to propylene oxide fumes prior to the experiment. Twenty-five grams of soil were put into a 250 mL beaker along with a 5 mL beaker containing 2 mL of propylene oxide. Three layers of plastic wrap were fastened over the mouth of the large beaker, and it was set aside for 48 hours.

In the first 24 hours, pressure generated inside the beaker domed the plastic wrap upwards, and by the end of 48 hours, the pressure had decreased and the plastic was pulled down into the beaker about a centimeter. The wrap was then removed and the soil was allowed to ventilate for a few hours. This reduced soil moisture, from about 21% down to about 13%, but not to air dryness, which is 5% in this soil.

Reaction

The reaction with oxalate was carried out at four concentration: zero, 100, 500, and 1000 μM of added oxalate, in duplicate. This was done using fumigated soil from each horizon, giving a total of sixteen reaction vessels. In addition, two unfumigated soil samples from the A1 horizon were reacted with zero added oxalate, and two unfumigated soil samples from the A2 horizon were reacted with 1000 μM added oxalate, both as controls for fumigation effects.

The reaction vessels were 500-mL polypropylene wide mouth, screw-top bottles, to which were added 25 g of soil and 280 mL of

deionized water. They were placed on an end-over-end shaker at 15 rpm for 18 hours, in order to bring the solutions to a quasi-equilibrium.

The first sample was taken at this time (t = 0 hours), using the procedure followed in all subsequent samplings: The pH of the solution was measured directly in the bottle with a pH meter, 9 mL of the solution was withdrawn and transferred to a polypropylene centrifuge tube, and the reaction bottle was returned to the shaker. After centrifuging the sample for 45 minutes, the supernate was decanted to a syringe fitted with a 0.45 µm nylon membrane filter. One-half milliliter of solution was filtered and discarded, and the balance was filtered into a 20 mL poly storage bottle, which was frozen until analysis.

Samples were taken at 0, 1, 3, 6, 11, 24, 48, and 76 hours for the A1 horizon, and at 0, 1, 2.5, 4, 9, 14, 21, 48, and 96 hours for the A2 horizon.

Before the bottles were returned to the shaker after the first sampling, the oxalate was introduced. Twenty milliliters of concentrated oxalate solution were added to each bottle, each solution containing the appropriate amount of oxalate to yield the desired concentration (i.e. 0, 100, 500, 1000 μM). The pH of the concentrated solutions was adjusted with NaOH, prior to mixing, in order to yield a reaction solution close to the pH of the natural soil.

Analysis

pH values were determined with a glass combination electrode. 3:1 water soil slurries were prepared and measured after a thirty minute

equilibration time. pH of the solutions during the reaction were measured directly in the reaction bottles.

Total soluble oxalate and orthophosphate concentrations were determined by HPLC on a Dionex 2000i. A preliminary study was conducted to verify that aluminum would not significantly affect the oxalate measurement (see appendix B). The detection limit for oxalate was 6 μM, and the detection limit for orthophosphate was 0.8 μM.

Monomeric aluminum was analyzed colorimetrically by reaction with 8-hydroxyquinolone, in a variation of the method of Bloom, et al. (1978) (see Appendix A).

Total dissolved organic carbon was measured by oxidation to CO_2 with a Dohrmann Carbon analyzer. The detection limit of this method was below 0.1 mmolC/L.

Solution speciation was performed by the MICROQL computer program (Westall, 1986). Input data included total solution concentrations of oxalate, aluminum, phosphate, sulfate, total dissolved organic carbon, and pH. The use of conditional stability constants throughout allowed the expression of results as concentrations. Speciation data for Al^{3+}, OH^-, and PO_4^{3-} were used to calculate the IAP's of aluminum hydroxide, $(Al^{3+})(OH^-)^3$ and aluminum phosphate, $(Al^{3+})(PO_4^{3-})$.

RESULTS

Propylene Oxide Fumigation

Treatment with propylene oxide slowed the disappearance of oxalate (Fig 3.1) and aluminum from solution. This is likely due to reduction

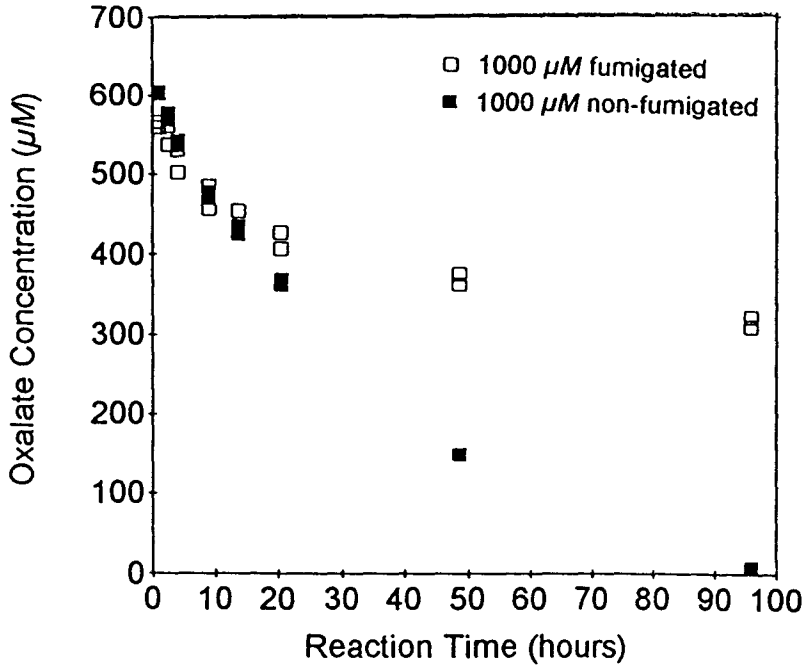
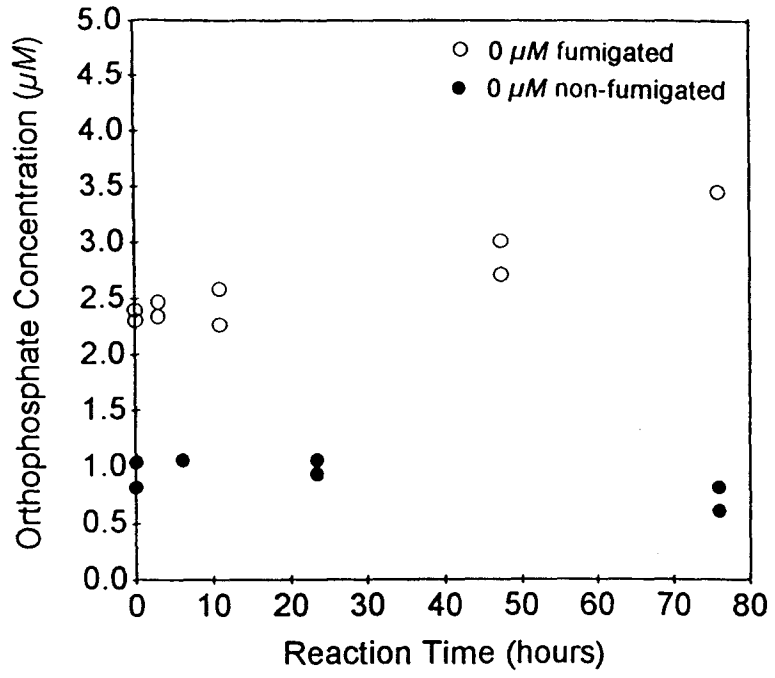
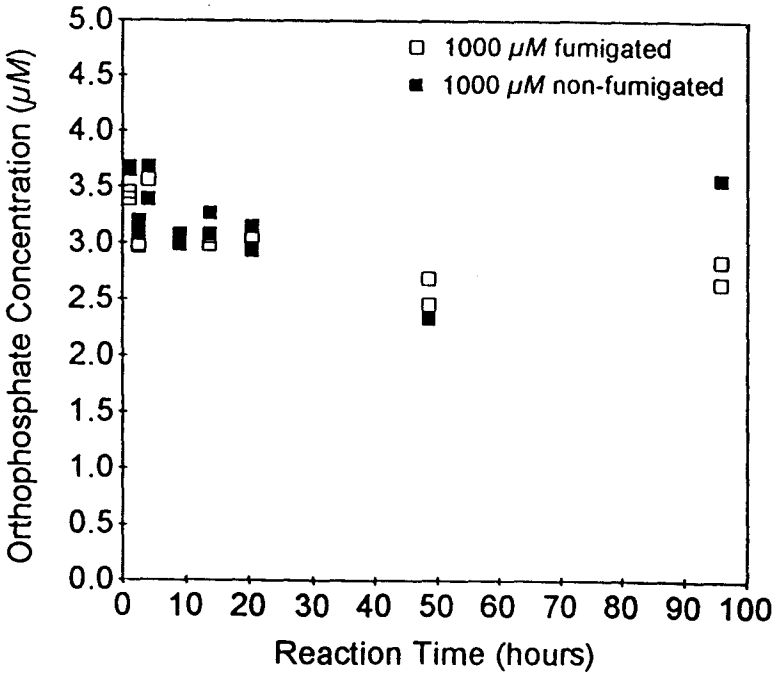


Fig. 3.1. Effect of fumigation on oxalate concentration.

in microbial numbers, slowing the metabolism of oxalate. Fumigation caused a slightly higher (+4%) initial oxalate concentration, and a slightly higher initial total aluminum. There was no significant effect from fumigation on total P in the 1000 *μM* oxalate treatment; however, in the zero oxalate treatment fumigation caused an increase in ortho P solubility (Fig 3.2). Fumigation had no effect on the IAP's of either gibbsite or aluminum-phosphate (Figs. 3.3 and 3.4) in the 1000 *μM* oxalate addition. When no oxalate was added, aluminum could not be determined with



A1 Horizon



A2 Horizon

Fig. 3.2. Effect of fumigation on orthophosphate concentration.

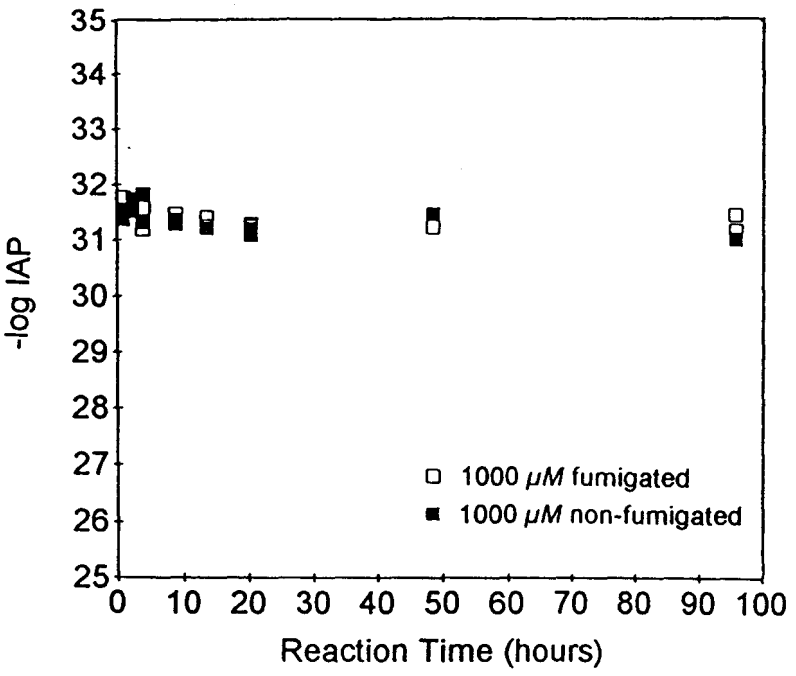


Fig. 3.3. Effect of fumigation on $Al(OH)_{3\ solid}$ Ion Activity Product.

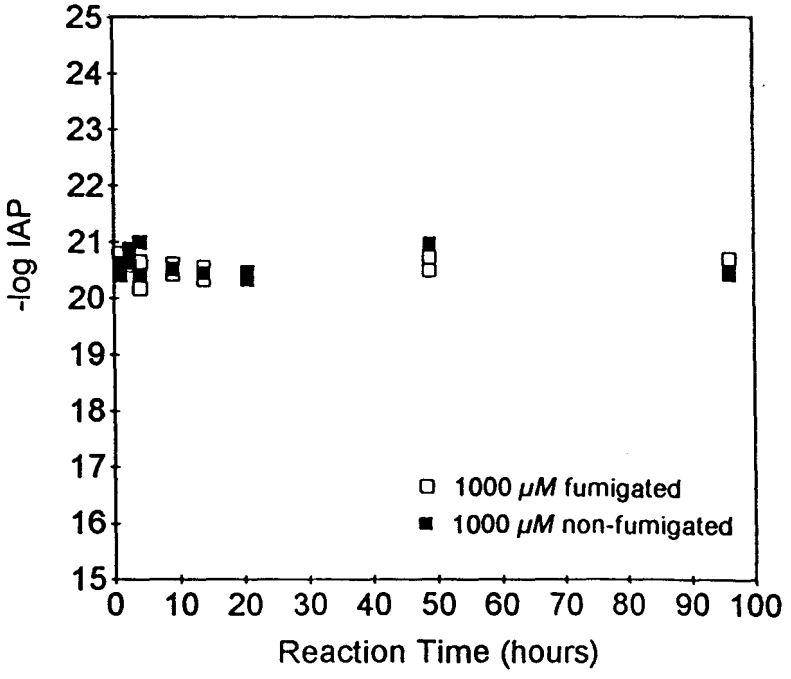


Fig. 3.4. Effect of fumigation on $AlPO_{4\ solid}$ Ion Activity Product.

sufficient accuracy to calculate an IAP. Soils that were treated with propoxide had a lower pH in 10:1 water:soil than did the untreated soils, by about 0.4 pH units.

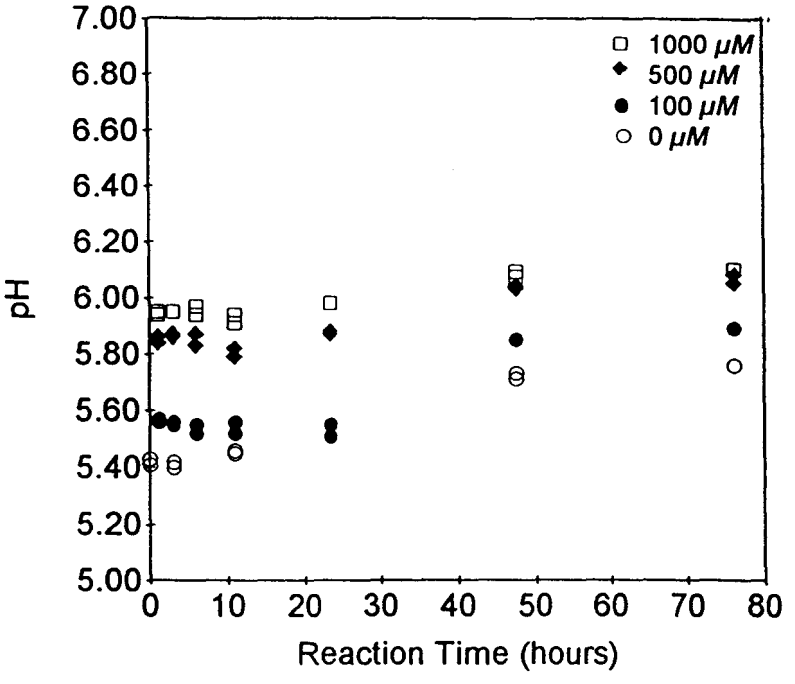
pH

The pH of the A1 and A2 horizons in 3:1 water:soil slurries were between 5.81 and 5.83, respectively. Sterilization with propylene oxide vapors result in pHs of 5.42 and 5.43. This decrease is the opposite of the results obtained by Skipper and Westerman (1972). The oxalate solutions were adjusted to pH 5.8 before addition to the reaction bottle. The 500 and 1000 *μM* treatments began at about pH 5.8, while the 100 μM treatment began at a lower pH of about 5.55.

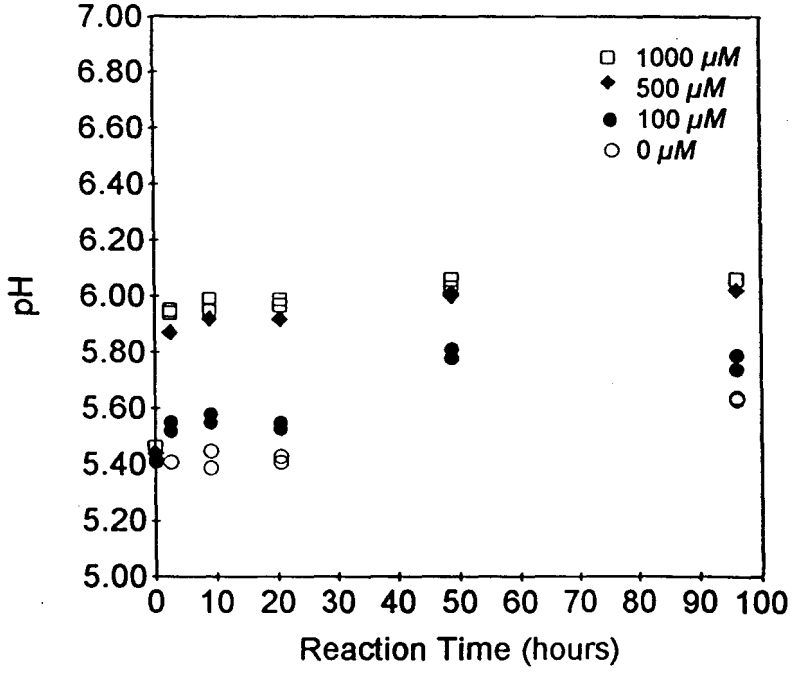
An upward trend in pH values was observed in all treatments, with the most prominent increase between 24 and 48 hours. The treatments with the most oxalate (which also had the highest pH) showed the least pH change (Fig 3.5).

Sulfate

In the high oxalate treatments in both horizons sulfate increased during the first 12 hours (Fig. 3.6). This concentration was maintained throughout the reaction in the A2 horizon, while in the A1 horizon the concentration slowly dropped to its original level. Sulfate concentration was approximately twice the concentration of phophate during the reaction.



A1 Horizon



A2 Horizon

Fig. 3.5. pH during the reaction for four levels of oxalate addition.

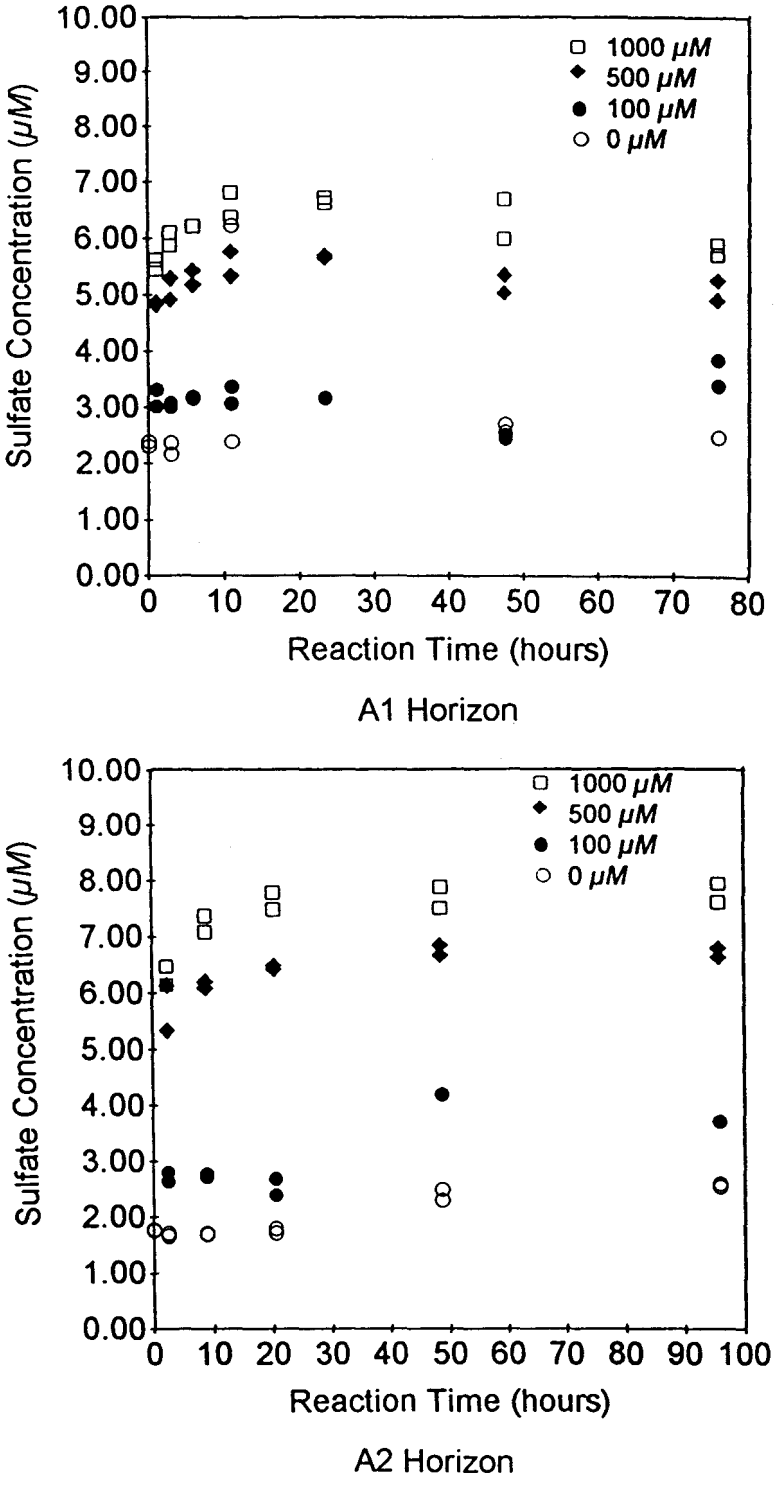


Fig. 3.6. Sulfate concentration during the reaction for four levels of oxalate addition.

Oxalate

A fraction of the added oxalate disappeared from solution within one hour in all treatments. This fraction was approximately 92% in the 100 *μM* treatments, 60% in the 500 *μM* treatments, and 43 % in the 1000 *μM* treatments. This initial rate of disappearance is markedly greater than the subsequent rates, and is greater than would be expected given the roughly exponential decrease in concentration exhibited in Fig. 3.7.

Phosphate

In the A1 horizon, total phosphate was increased by larger additions of oxalate, from approximately 2 *μM* with zero oxalate, to 4 *μM* with 1000 *μM* oxalate. These concentration were maintained, and slightly increased, for the first twenty four hours, after which time, one half of the repetitions declined, while the others remained high (Fig 3.8). The level of oxalate treatment had no bearing on which repetition showed a disappearance of P.

In the A2 horizon, the same pattern of response to initial oxalate addition was observed, but the range of P concentration was lower (from about 0.9 to 3.4 *μM* P). There was also a slight decrease in concentration in all treatments (Fig 3.8).

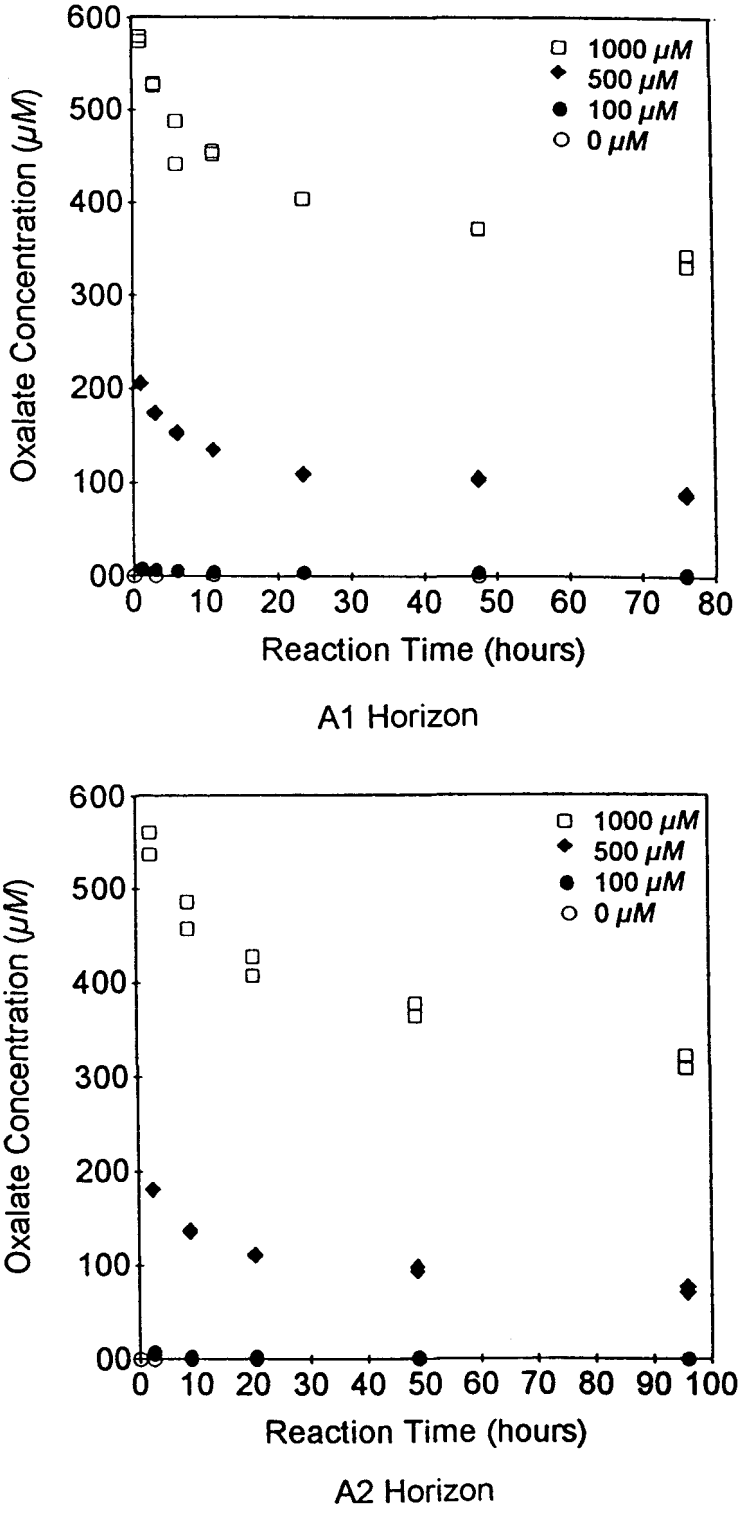


Fig. 3.7. Oxalate concentration during the reaction for four levels of oxalate addition.

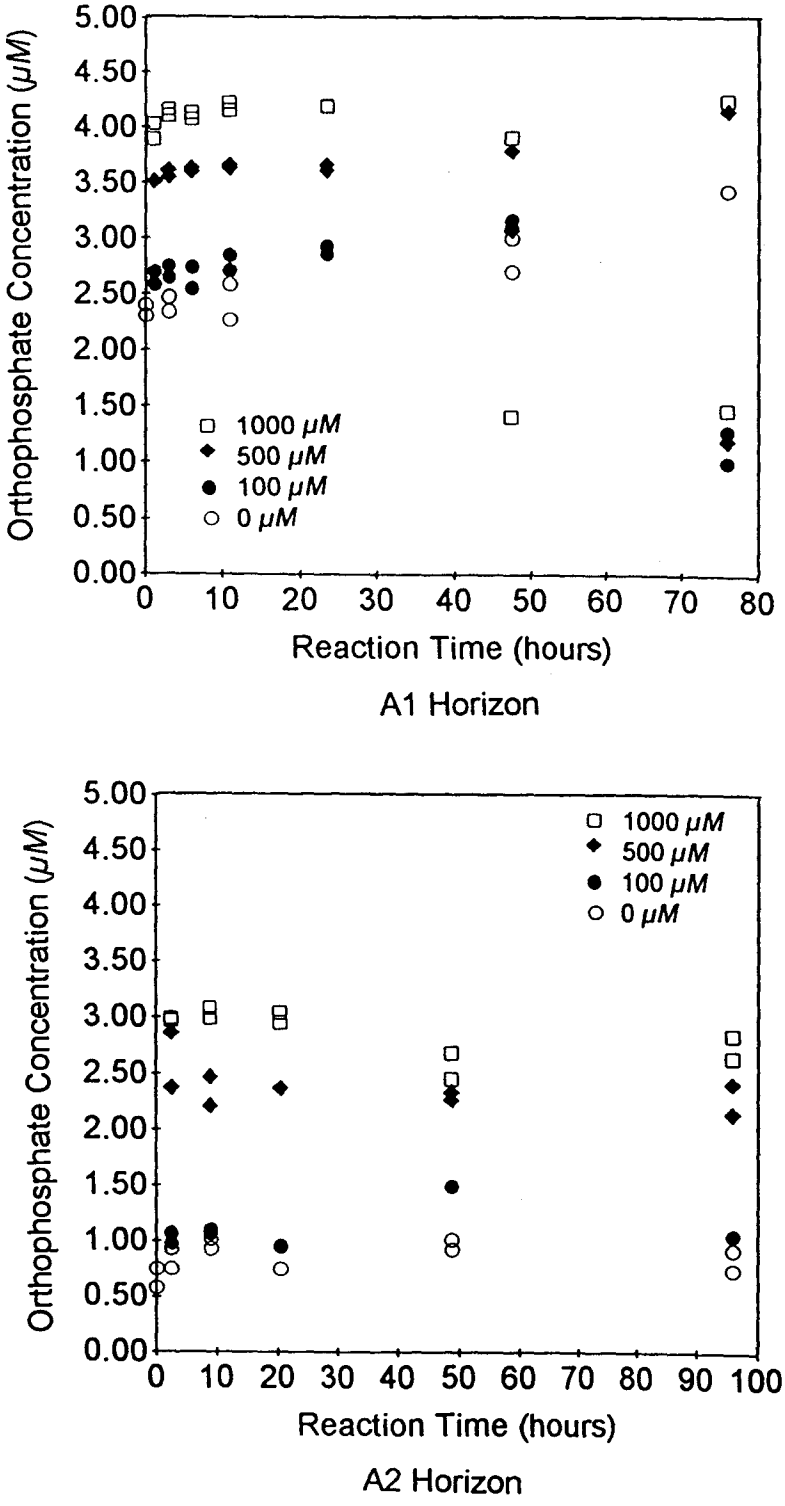


Fig. 3.8. Orthophosphate concentration during the reaction for four levels of oxalate addition.

Aluminum

The solution concentrations of monomeric aluminum were closely related to the concentration of oxalate. A total oxalate:total monomeric aluminum ratio at all times during the reaction was equal to 3, when the aluminum was above the limit of detectability. The 500 and 1000 *μM* oxalate additions caused a large initial increase in aluminum, followed by a slow decrease to values which were still much higher than before addition (Fig 3.9).

Carbon

The concentrations of total dissolved organic carbon were 300 μmolC/L in the untreated A1 horizon solution, and 200 μmolC/L in the A2. Addition of 1000 μM oxalate to the A2 increased TOC to 600 μmolC/L. This data was used in the speciation program with the following assumptions: The initial non-oxalate C is one half fulvic acid and one half non-complexing compounds (alcohols, weak acids), and this initial fulvic acid remains constant throughout the experiment; the fulvic acid is chemically similar to the Suwanne River FA described by Drever (1988); all of the carboxyl groups, and none of the phenolic groups, are dissociated; the association constant of Al with this fulvic acid is $10^{8.55} = [AlL]/[Al^{3+}][L^{3-}]$ (Blaser and Sposito, 1987).

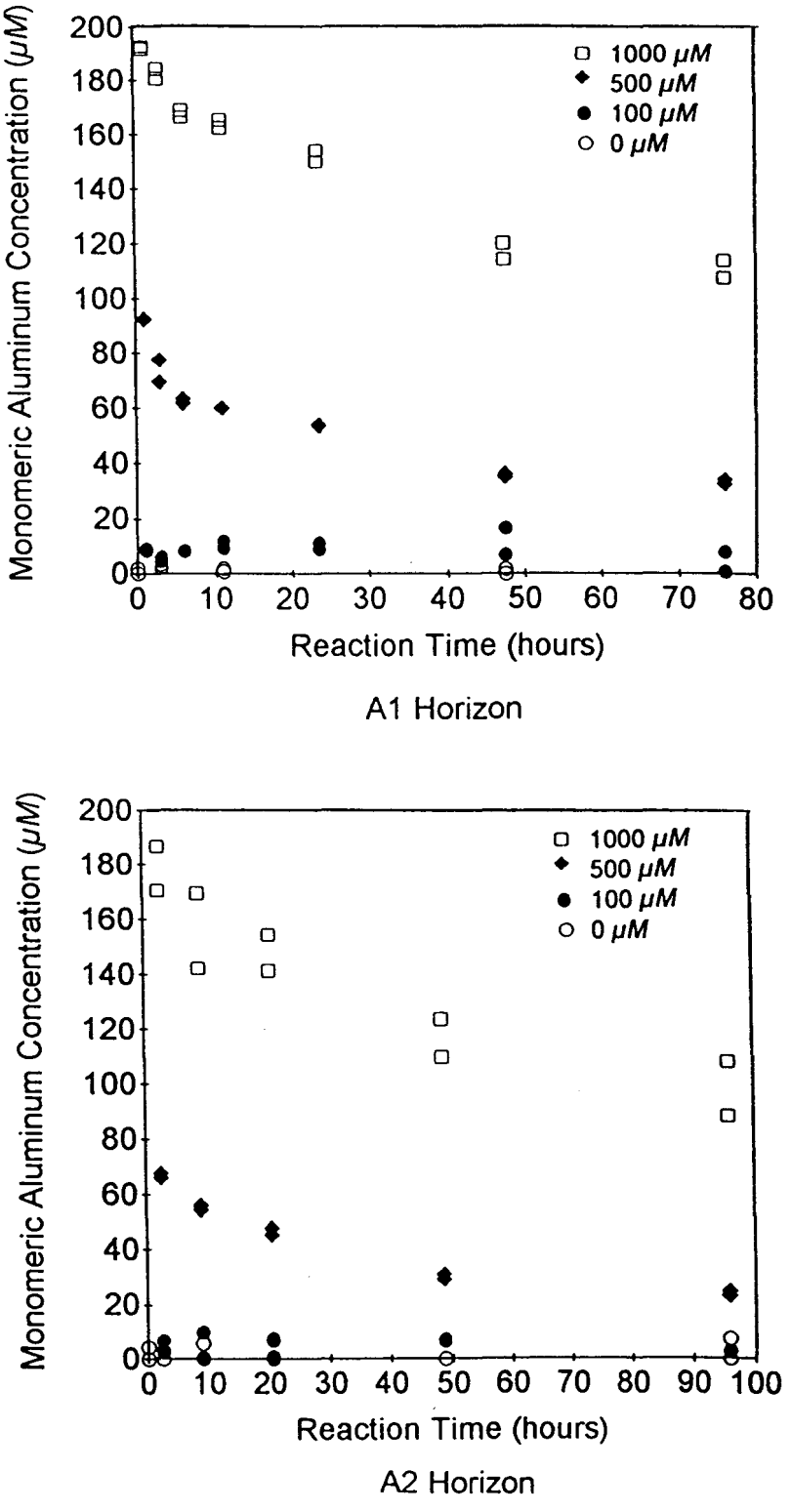


Fig. 3.9. Monomeric aluminum concentration during the reaction for four levels of oxalate addition.

Ion Activity Products

MICROQL speciation data for Al^{3+}, OH^-, and PO_4^{3-} were used to calculate the IAP's of aluminum hydroxide and aluminum phosphate.

In both horizons and for both 500 and 1000 *μM* additions, the $Al(OH)_{3\ solid}$ IAP, $(Al^{3+})(OH^-)^3$, was steady at about $10^{-31.25}$ (Fig. 3.10). Al^{3+} activity remained essentially constant while total monomeric aluminum decreased by 50 %.

The IAP of aluminum phosphate, $(Al^{3+})(PO_4^{3-})$, was not different between the 500 and 1000 *μM* treatments. In the A1 horizon, the IAP showed a slight increase over the course of the reaction, from $10^{-20.25}$ to $10^{-20.5}$, while in the A2, there was no trend (Fig. 3.11). These IAPs lie at the equilibrium level for amorphous Al-P (Cole and Jackson,1950; Taylor and Gurney,1962), and are quite supersaturated with respect to variscite.

Because of the inherent inaccuracy in the estimation of soluble organic ligands and the low concentrations of total aluminum, numerical speciation did not yield $[Al^{3+}]$ values of sufficient accuracy to calculate valid IAP's in the low oxalate treatments. Ion activity products for the zero and 100 *μM* additions are presented and plotted but are not to be considered reliable data.

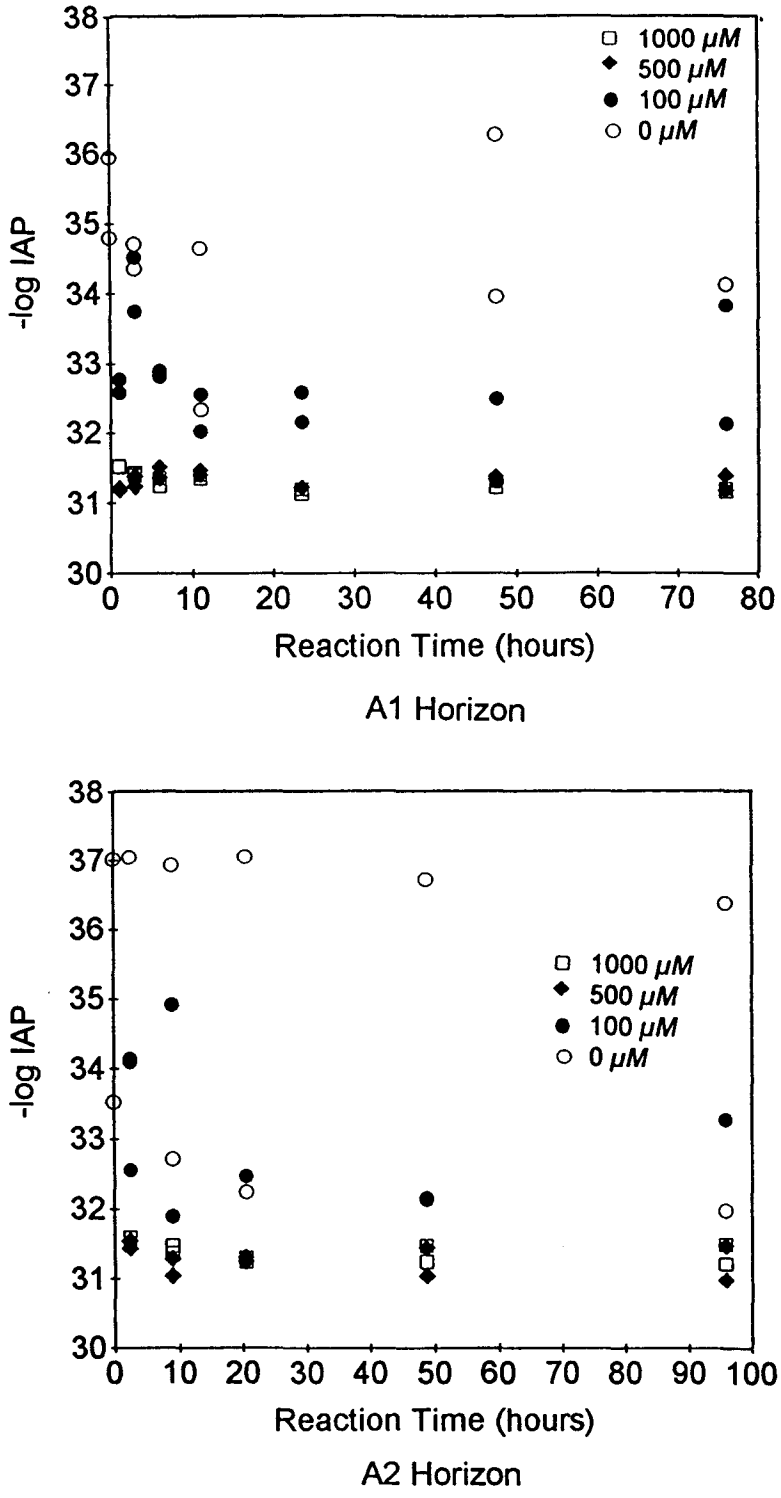


Fig. 3.10. Aluminum hydroxide ion activity product, $(Al^{3+})(OH^{-})^3$, during the reaction for four levels of oxalate addition.

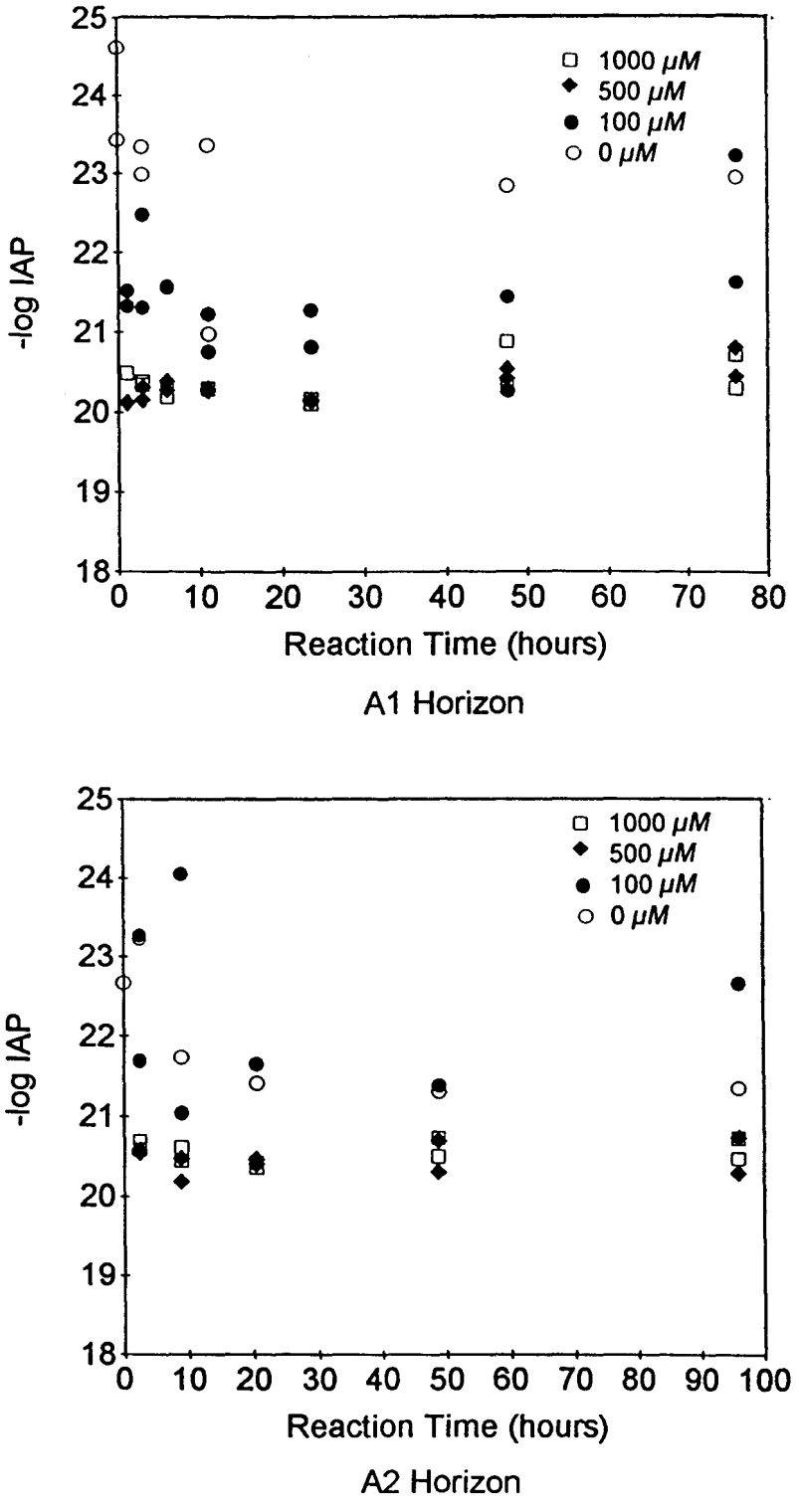


Fig. 3.11. Aluminum phosphate ion activity product, $(Al^{3+})(HPO_4^{3-})$, during the reaction for four levels of oxalate addition.

DISCUSSION

Oxalate

Though low molecular weight organic anions such as oxalate are readily incorporated into aluminum and hydrous oxides during their formation, the decrease in solution oxalate can not be primarily attributed to this mechanism. Oxalate loss by precipitation exceeds the amount of aluminum lost from solution to such an extent that any aluminum phase formed could not contain the oxalate.. Adsorption also cannot account for the oxalate decrease because there is no apparent mechanism for this adsorption to continue throughout the entire reaction period. The difference between oxalate added and oxalate measured at one hour should be attributed to adsorption. Either microbial activity or oxidative, photochemical reactions are likely causes for the observed decrease (see Chapter 2, Standard Stability). In non-fumigated soils, microbial degradation of oxalate may predominate, while in fumigated soils abiotic factors may be of more major importance (Fig. 3.1), though it is unlikely that microbial activity was completely inhibited.

Aluminum

The aluminum that entered solution at the start of the reaction was present mainly as $Al(Ox_2)^-$ and $Al(Ox_3)^{3-}$, indicating that the oxalate was involved in the release of aluminum. Possible sources of this aluminum include desorption, release of interlayer aluminum, and dissolution of aluminum-containing solid phases such as clay minerals and aluminum hydroxides and phosphates. Desorption of aluminum induced by reduction

in solution-(Al^{3+}) by oxalate is likely, because speciation calculations indicate a decrease in (Al^{3+}) with greater additions of oxalate. Also, the Na^{+} derived from the pH adjustment of the oxalate solutions will also promote the desorption of Al^{3+}. At the rate of gibbsite dissolution at pH 1.9 determined by Bloom and Erich (1986), solution aluminum would only reach 4 *μM* in the first hour of reaction. The presence of oxalate will accelerate this process, though the higher pH in the present study will hinder it. It appears that this is not a major source of the aluminum. The dissolution kinetics of amorphous materials are quite a bit faster than those of highly regular solids, and it is conceivable that these solid phases made a large contribution to the initial flush of aluminum.

As oxalate disappeared from solution, there was no delay in the decrease in solution aluminum, suggesting that the there is no kinetic constraint for this process. Three possible final forms for this aluminum (explaining the decrease in solution-Al during the reaction) are amorphous aluminum phosphates, amorphous hydrous aluminum oxides (or some mixture of the two), or exchangeable aluminum. Because phosphate remains elevated throughout the reaction and its concentration is so much less than that of aluminum, the formation of significant aluminum-phosphate solid can be ruled out. The observed increase in pH in the latter half of the reaction casts doubt on the role of $Al(OH)_3$ precipitation. The formation of $Al(OH)_3$ from an Al-oxalate complex will consume OH^-, lowering solution pH:

$$Al(Ox)_2^- + 3OH^- = Al(OH)_{3\ solid} + 2Ox^{2-}$$
$$AlOH(Ox)_2^{2-} + 2OH^- = Al(OH)_{3\ solid} + 2Ox^{2-}$$
$$Al(Ox)_3^{3-} + 3OH^- = Al(OH)_{3\ solid} + 3Ox^{2-}$$

Because oxalate-Al is no more hydrolyzed than exchangeable-Al, the effect on pH from the readsorption of aluminum should be minimal.

The IAP for $Al(OH)_{3\ solid}$ is steady throughout the reaction for the high oxalate treatments and is consistent with solution-Al control by a hydrous aluminum oxide such as gibbsite or interlayer $Al(OH)_3$. It may be that this is the long term position of aluminum solubility in this soil, and that transformations of aluminum due to short term perturbations involve only the exchangeable aluminum pool.

Phosphate

The release of phosphate at the start of the reaction could be due to the rapid dissolution of amorphous aluminum-phosphate solids, to the dissolution of aluminum hydroxide exchanger phases, or to displacement from exchange sites through ligand exchange with oxalate. The previous discussion suggests that the first two mechanism are not significant. A clear correlation between the two divalent species in all treatment (Fig. 3.12) illustrates the competition between the two anions. When oxalate is high, it displaces more phosphate from the exchanger phase into solution.

The observation that phosphate does not decrease during the reaction period is further argument against the formation of amorphous $Al(OH)_3$ in the last half of the reaction. This solid phase is a very strong sorbent for P, especially when formed in the presence of organic anions (Ng Kee Kwong and Huang, 1975), and if present would be expected to cause a decrease in phosphate.

Sulfate

The behavior of sulfate lends support to the view that P is released through anion exchange. Sulfate is increased sharply upon introduction of oxalate, and then curiously continues to increase slowly for the next 12 hours. The adsorption affinity of sulfate is low compared to that of oxalate or phosphate, and does not compete well for exchange sites. However, higher oxalate additions displaced more sulfate than lower additions, which indicates that at low additions, some sulfate remained on the exchange sites. Sulfate is able to retain its adsorbed position, but is less able to displace a pre-existing anion (Violante, et al., 1991); this may account for the slow rise in sulfate, as the lingering sulfates are replaced by the anions with greater sorption affinity.

CONCLUSIONS

The addition of oxalate to this soil had a major effect on both aluminum and phosphorus solubility, causing an increase in both. Oxalate appears to function in two important ways: the first is the displacement of phosphate from exchange positions, and the second is the reduction of Al^{3+} activity by the formation of soluble Al complexes. The elevation of solution phosphate due to desorption could not be maintained in the presence of baseline aluminum levels, because this would result in a supersaturation with respect to solid aluminum phosphate. Oxalate's

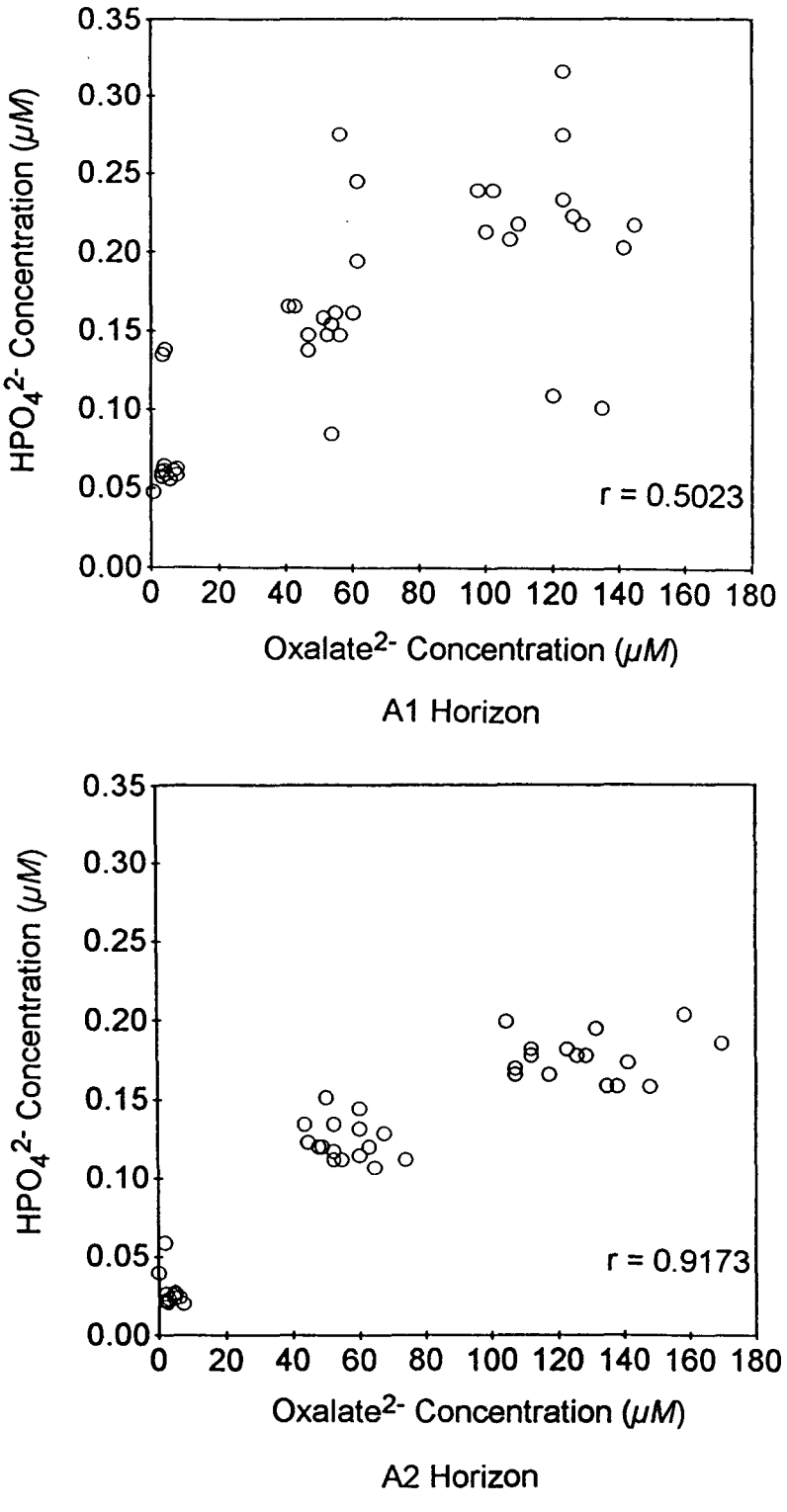


Fig. 3.12. Relationship between HPO_4^{2-} and $oxalate^{2-}$ for all treatments.

sequestration of Al through complexation ensures that soluble phosphate is maintained.

The source of the aluminum solubilized by oxalate cannot be positively determined, but the data suggests that it is mostly derived from the exchangeable pool. This conclusion is supported by the fast kinetics of the reaction, both initially when solution Al increases, and later when Al concentration parallels the concentration of oxalate. An important feature of the aluminum distribution is that though total solution Al increases greatly, it is mostly present in organic complexes, and the phytotoxic species Al^{3+} is maintained at a low concentration.

After 96 hours of reaction, solution phosphate remained elevated and, except in a few cases, showed no decrease at all. The replicates in which P concentration dropped do not show a pattern and may be an experimental artifact, perhaps biological. That P remains high even when more than half of the added oxalate has disappeared is a surprise, and indicates that production of oxalate in soils may have lasting effects. Oxalate may persist on the anion exchange sites, hindering the readsorption of phosphate. In a soil in which oxalate production is sustained, further changes involving solid phase aluminum should become important.

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CHAPTER 4
SUMMARY and PROJECTIONS

With the support of ion chromatography analysis, a batch reaction of a low molecular weight organic acid with soil yielded meaningful information on soil processes. Useful laboratory studies in soil chemistry depend upon reliable methods for measuring soil solution parameters, as well as upon techniques for faithfully reproducing natural field reactions in a controlled setting. When information is drawn from laboratory studies, care must be taken to detect artifacts of experimental design that do not correspond to real soil processes. The first study in this thesis presented reliability data for a promising analytical method for low molecular weight organic acids. This method was applied in the second study, in which the effect of a particular acid, oxalic, was reproduced in the laboratory.

As a guide to future work, a few aspects of the study in which further refinement of technique would be beneficial will be mentioned. The "side effects" of the chosen biostat introduce complications into the interpretation of results, particularly the effects on pH and carbon dynamics. Also, because of their affinity for oxalate, inclusion of calcium and iron data will give a more complete model of oxalate's behavior. A more extensive chemical characterization of the subject soil also would assist in result interpretation.

The effects of oxalate demonstrated here have important implications for plant nutrient availability and for pedogenesis. The local elevation of solution P and reduction in Al^{3+} contribute to greater soil

fertility. To the extent that these effects are not local and not restricted to the immediate vicinity of the mycorrhizae, mineral weathering and leaching will be accelerated. Soil management would benefit from a more complete inventory of the occurrence of oxalate in both natural and agricultural systems. These are all areas in which a more detailed understanding of oxalate dynamics will reap rewards.

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APPENDICES

APPENDIX A

The Determination of Aluminum in Soil Solutions

Considerations

The total concentration of aluminum in solution can be measured by atomic absorption or plasma spectrometry, but in order to study the metal's influence on plant growth or on mineral solubility, we need to distinguish the different forms of aluminum that together compose the total. In particular, the hexaquoaluminum ion, Al^{3+}, must be considered, for this ion is directly involved in mineral solubility and plant root growth. However, it is difficult to obtain an accurate measure of just this one species in soil solutions, and usually an indirect method is used. The computational method can be used if the following conditions are met: the concentrations of all Al complexing ligands and their association constants are known (which requires knowledge of the associational relations of these ligands with the other elements with which they interact); the association of Al with fulvic and humic acids can be specified or if solution organic carbon is very low; and a total-Al value can be obtained which does not include polymeric species. These conditions being satisfied, a computer program such as MICROQL can determine the speciation of Al and of all solution components. Because of uncertainty in these conditions, however, a direct method for the determination of monomeric Al would be preferred.

Luster et al. (1993) found that a spectrophotometric method involving a 15-second reaction with 8-hydroxyquinoline measured monomeric aluminum species and did not measure polymeric forms. They did not investigate the effects of complexing ligands. James et al. (1983)

indicated that significant amounts of citrate- and fluoride-Al were measured by a 15-second reaction; and that these complexes were increasingly included as reaction time was increased. Okura et al (1962) used a shorter reaction time, in which aluminum and 8-hydroxyquinoline reacted at the same time that the complex was being extracted into chloroform. Turner (1968) found evidence that the method of Okura et al. measured Al^{3+}, $AlOH^{2+}$, and $AlOH_4^-$, and excluded polymeric species. Thus, it is possible that this shorter reaction time may result in the measurement of only monomeric species, with the exclusion of polymeric and complexed species.

A standard curve generated from the analysis of $AlCl_3$ solutions by a 15-second reaction with 8-hydroxyquinoline had an r^2 of 0.998 and a detection limit of 6 μM. Test solutions containing various amounts of $AlCl_3$ and oxalic acid were analyzed. In all but one case, there was an overmeasurement of mono-Al. The most serious overestimations occurred in solutions in which total oxalate exceeded total aluminum (in which solutions the mono-Al concentrations were well below 1 μM). The relative error in the determination increased sharply as the total Ox:total Al ratio approached 1. Apparently, a significant fraction of the oxalate-Al was measured by the method. Only when oxalate is less than half the concentration of aluminum is this method useful for the determination of monomeric, uncomplexed aluminum.

Because oxalate predominates in the present experiment, the indirect, computational approach was used. Total monomeric aluminum

was measured colorimetrically by a 1.5 hour reaction with 8-hydroxyquinoline, in a method similar to Bloom, et al (1978).

Method

Reagents:

- 200 g/L Hydroxylamine Hydrochloride (to reduce Fe^{3+} to Fe^{2+}).
- 1M Sodium Acetate with 0.2% 1,10-phenanthroline (for pH buffering and complexation of reduced iron) - Dissolve 2 g phenanthroline with heating in 800 mL water. Cool, add 82 g sodium acetate, and dilute to 1 L.
- 1% 8-hydroxyquinoline (to form the colored complex with Al^{3+}) - Dissolve 5 g 8-hydroxyquinoline in 12.5 mL glacial acetic acid, and dilute to 500 mL.
- Butyl Acetate
- Mixed reagent - The first three reagents mixed with water in the following proportions: Hydroxylamine-3%; 8-hydroxyquinoline-12%; Sodium acetate-15%; water-70%. This must be prepared daily.

Procedure

Add 20 mL mixed reagent and 3 mL of sample to a 50 mL polypropylene centrifuge tube. Cap and shake to mix, then let stand for 1.5 hours, to allow the reaction of Al with the 8-hydroxyquinoline. Add 4 mL of butyl acetate, shake vigorously for 30 seconds, and allow the phases to

separate for 30 minutes. Within a few hours, measure the absorbance of the organic phase at 395 nm against a butyl acetate blank.

The detection limit for this method is 4 μM aluminum.

APPENDIX B

Determination of Oxalate in Soil Solutions

In a high pH IC system, oxalate occurs as a bivalent anion, and conductivity detection returns a measurement of the total concentration of oxalate. Oxalate's proclivity to form complexes with metal ions raises the question of whether this occurs on the HPLC column, and if so, do these complexes have different retention times or different conductivities than the Ox^{2-} anion? In the context of the present experiment, will aluminum interfere with the analysis of oxalate?

To answer this question, samples of oxalate solutions were made up and aluminum was added to them at different rates. A slight reduction in measured oxalate was found as more aluminum was added. At equimolar concentrations, a 4% decrease occurred, and when total aluminum approached twice the oxalate concentration, the reduction exceeded 6%. For the purposes of this study, in which the Al:Ox ratio is well below 2, this accuracy is deemed acceptable. However, in other studies, this effect should be recognized.

APPENDIX C

Detection Limits

The detection limit for an analytical method expresses the lowest concentration of analyte that can be detected with confidence. It has been defined as that amount of analyte producing a signal twice the average amplitude of the noise (Small, 1989), or twice the background fluctuation (Manahan, 1979), or three times the standard deviation of the baseline signal. (Howe et al., 1991), or three times the standard deviation of a low concentration standard (Franson, 1995). These are all statistically justified; however, when they are used with linear calibrations, these methods do not consider variability in the best fit line itself. By constructing confidence intervals for the prediction of concentration from instrument response, one can generate a detection limit which is well defined, easily calculated, and has a firm statistical basis.

First, a standard curve is generated using a concentration range close to the expected detection limit. Then, we can determine the 99 % confidence limits for the prediction of Y, instrument response, from any given x:

$$Y_{bar} + bx \pm t \cdot s_{y.x} \cdot \sqrt{1 + \frac{1}{n} + \frac{x^2}{\sum_{n} x^2}}$$

where x = deviation from the average of standard concentrations X (X_{bar}), Y_{bar} = average of standard responses Y, b = regression slope, $s_{y \cdot x}$ = sample standard deviation from regression (Snedecor and Cochran, 1967), n = number of standards, and t = t-test value for 99% confidence. At $x = -X_{bar}$ (i.e., concentration = zero), we find the confidence limits at the y-axis, and the upper limit (point M, Fig. C.1) is:

$$Y_{bar} - bX_{bar} + t \cdot s_{y.x} \cdot \sqrt{1 + \frac{1}{n} + \frac{x^2}{\sum x^2}} = Y_M$$

where Y_M is the response value at point M, where concentration = 0. Point M is also the lower confidence limit for the prediction of x from Y_M. Furthermore, Y_{upper} is the lowest value of Y, instrument response, from which a non-zero concentration can be predicted with 99% confidence. This response describes the detection limit (point C in Fig. C.1). To express this as a concentration, transform the expression through the regression equation $(Y-Y_{bar})/b = x$ (recalling that $x+X_{bar}$ = concentration) to yield:

$$\frac{t \cdot s_{y.x} \cdot \sqrt{1 + \frac{1}{n} + \frac{X_{bar}^2}{\sum x^2}}}{b} = \text{detection limit}$$

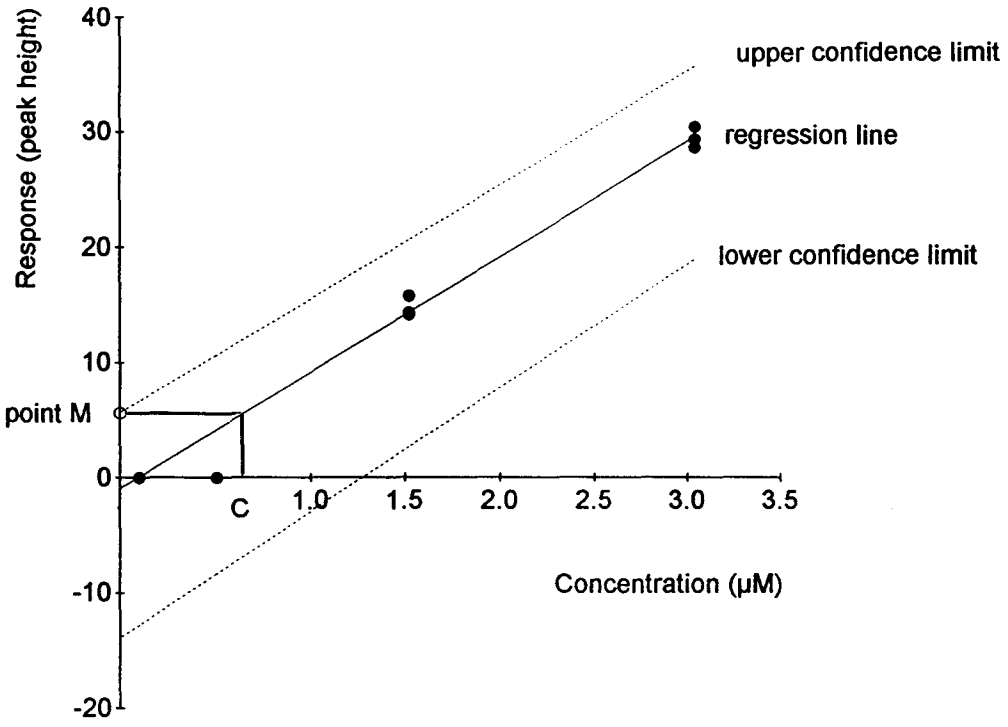

Fig. C. Determination of a detection limit from the lower end of a standard calibration curve (where point C is the detection limit).

APPENDIX D

Soil Sterilization

In order to restrict the processes in this experiment to the abiotic, and especially to discourage the decomposition of oxalate, methods for the inhibition of biotic activity in soil samples including autoclaving, toluene addition, azide treatment, fumigation by chloroform and by epoxides were considered.

In their comparison of antibiotic methods, Skipper and Westermann (1973) found significant effects from autoclaving on extractable Mn, and increases in extractable P. In addition, the heat involved in autoclaving causes transformations in aluminum and iron solids (Schwertman and Taylor, 1989).

Toluene was used as a biostat in the study of Fox et al. (1990), and was found to be inadequate after 24 hours.

The azide ion, introduced into solution as a sodium or potassium azide, is an effective enzyme inhibitor, disabling them by binding with metal atoms in the enzyme structure (Mangani and Orioli, 1992). Thus it is necessary that the azide ion be present in solution at all times that biostasis is desired. However, the tendency of azide to complex metals is undesirable in the present investigation, which is specifically concerned with metal complexation by oxalate. Calculations show that azide competes too well for complexation with Al and Fe and would be an unacceptable interference with the activity of oxalate in the present study.

Chloroform fumigation was investigated by Jenkinson and Powlson (1976) in order to develop a method for microbial biomass estimation. They found that most of the microbes were killed by the treatment, and in subsequent incubation CO_2 was evolved at a greater rate than in non-fumigated incubated soils - a phenomenon they called "flush". The flush was the same in fumigated samples whether they were inoculated with fresh soil before incubation or not. They concluded that the flush was due to the metabolism of carbon from cells lysed during fumigation, and that there was sufficient survival of microbes to produce the flush.

In a second experiment (Powlson and Jenkins, 1976), the magnitude of the flush was much less in acid soils than in neutral soils, apparently because of the lower microbial population in the former.

Ethylene and propylene oxide are epoxides with a reactive oxygen atom, which reacts with organic functional groups containing labile protons, such as carboxyl and sulfhydryl groups, forming esters (Fraenkel and Conrat, 1944). These reactions are very effective in killing microbes, and a soil can be virtually sterilized by exposure to the vapors of these epoxides (Ark, 1947; Westermann et al., 1971). Treatment of a soil with ethylene oxide also resulted in a flush of CO_2 generation, but the onset of the flush was not observed until after two days of incubation.

Skipper and Westerman found no significant effect from propylene oxide treatment of the acid Jory soil on available P, extractable Mn, exchangeable K, or extractable N. However, soil pH was increased 0.5 pH

units. The previously mentioned transformations of acidic functional groups to esters causes an increase in the isoelectric point of the organic material, and presumably a consequent increase in solution pH.